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CRYSTALLINE COMPOSITION OF EQUINE URINARY SABULOUS DEPOSITS

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Abstract

The composition and crystal morphology of 141 equine sabulous deposits were determined by infrared spectroscopy (IR), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). The IR analysis revealed that all investigated deposits contained calcium carbonates (calcite, $CaCO_3$, and/or vaterite, $CaCO_3$) as major constituents; 42 samples were composed of calcite and vaterite, 33 of calcite, 18 of calcite/vaterite and calcium oxalate, and 17 of vaterite. The remaining specimens contained calcite/vaterite and other compounds (calcium phosphates, sulphate and/or oxalates and/or silica). The examination of 44 selected samples by means of SEM/EDX, revealed the characteristic morphology and elemental composition of the constituents of the sabulous deposits. Calcite crystals showed a typical spherical shape, as well as other less common rhombohedral habit. Vaterite displayed a "flower" or "star" appearance and also a "mulberry" shape. Less frequent was a spherular habit for vaterite resembling that of calcite. Elemental analysis of both calcite and vaterite crystals showed, besides calcium, varying proportions of magnesium and potassium. It is concluded that calcite and vaterite were mainly present in a substituted form. Calcium oxalate dihydrate crystals showed their characteristic bipyramidal morphology. Calcium oxalate monohydrate crystals, which were less frequent, exhibited "dumbbell" or "hour-glass" shape.

Key Words: Scanning electron microscopy, energy dispersive X-ray analysis, crystalline composition, infrared analysis, equine urolithiasis, equine urinary sabulous deposits, substituted calcite and vaterite, calcite, vaterite, calcium oxalates.

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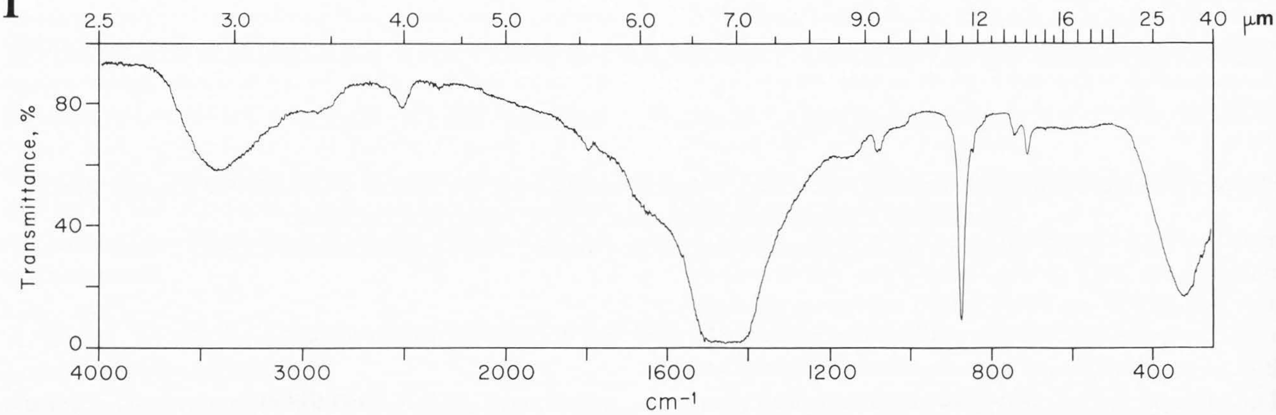
Introduction

Urolithiasis occurs infrequently in the equine species (incidence less than 0.5%) [4, 13, 22]. This fact seems surprising since the characteristics of normal equine urine make it an ideal medium for stone formation. These equine urine characteristics include abundant crystalluria (mainly calcium carbonate crystals although calcium oxalates and calcium phosphates can also be present), organic material (mainly mucoproteins), and pH ranging from 6.8 and 8.4. Moreover, equine urine normally has a large amount of calcium carbonate crystals, which are primarily responsible for its characteristic turbidity [8, 10, 14]. Additionally, normal equine urine is viscous due to the presence of mucus secreted by cells in the renal pelvis [10, 23]. In adults, the urine specific gravity ranges from 1.02 to 1.05 (mean 1.035) [23]. Renal excretion of calcium is important in equine calcium homeostasis, and horses can excrete very large calcium loads in concentrated urine [12]. Equine calculi usually form as discrete concretions of different sizes, commonly lodged in the bladder or urethra and less frequently found in the renal pelvis and ureters. Sabulous deposits appear as a doughy sludge, normally in the ventral bladder. The theory that these deposits can occur as a secondary feature to bladder paralysis has been previously postulated [14, 18, 20], and the amount of sediment has been related to the duration of the disease [1, 7, 8].

Using chemical methods, calcium carbonate has been revealed as the major constituent of sabulous deposits [1, 7, 8]. To date, there have been few reports concerning the composition and morphology of these urinary deposits [5, 6, 14, 17].

In the present work, the mineral composition of 141 samples of equine sabulous material has been examined by infrared spectroscopy (IR). From these samples, 44 have also been studied under scanning electron microscopy (SEM) and by energy dispersive X-ray (EDX) microanalysis. In the following, the analytical characteristics and crystal morphology of the main components (calcite, vaterite and calcium oxalate) are described.

1



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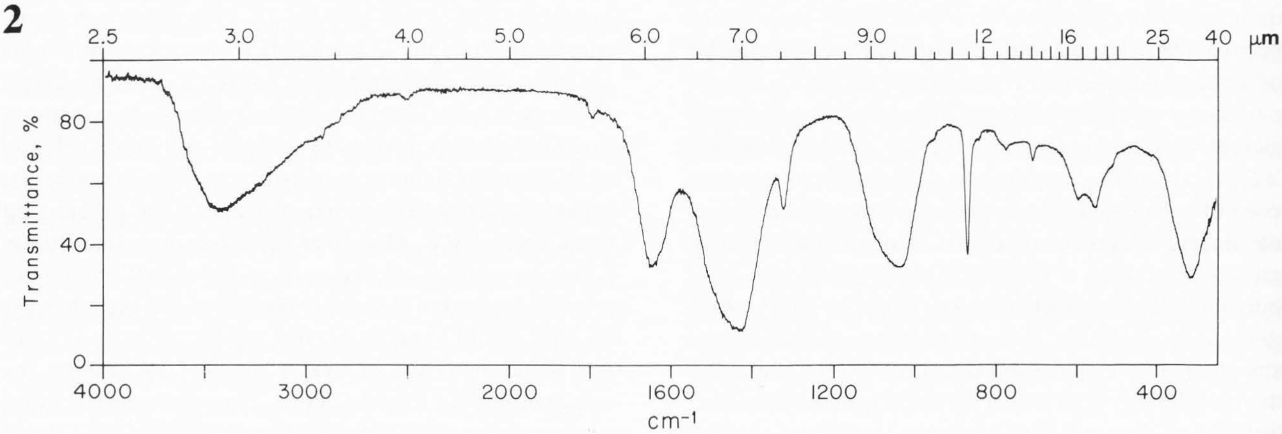


Figures 1 and 2. Infrared spectra of samples of sabulous material showing characteristic bands of calcite, vaterite, sulphate and phosphate (**Figure 1**), and calcite, calcium oxalate and calcium apatite (**Figure 2**). See text for details.

Table 1. Composition of 141 equine sabulous deposits determined by IR analysis.

Composition	Number of samples (%)
Calcite	33 (23.4 %)
Vaterite	17 (12.1 %)
Calcite and Vaterite	42 (29.8 %)
Calcite/Vaterite and Calcium Oxalate	18 (12.8 %)
Calcite/Vaterite and Calcium phosphates, sulphates and/or oxalates	25 (17.7 %)
Calcite/Vaterite and other compounds (calcium phosphates, sulphate, and/or oxalates and/or silica)	6 (4.3 %)

Materials and Methods

The majority of the samples (140) were collected from the bladders of 2025 slaughtered animals (1073 horses, 521 donkeys and 431 mules). One sample was obtained from the renal pelvis. The samples were washed with distilled water, filtered and dried at room temperature. Usually, two or three samples from the same specimen were analyzed by means of IR analysis. From a macroscopical point of view, the deposits can be considered rather homogeneous, although quantitative results were different when samples were examined under SEM/EDX .

The infrared spectra of the sabulous deposits were recorded with a Perkin Elmer 599B spectrophotometer (4000-200 cm^{-1}) using the KBr technique (ca. 1 mg sample: 300 mg KBr).

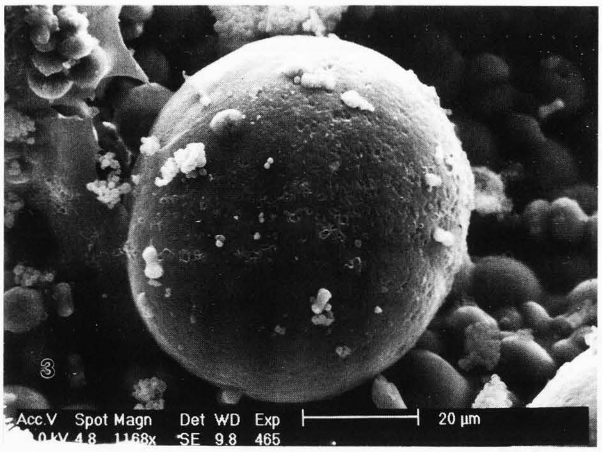
The crystal morphology and elemental composition of the samples (covered with gold in a sputter coater) were studied with a Philips SEM XL30, equipped with a PV 9900 EDX analyzer.

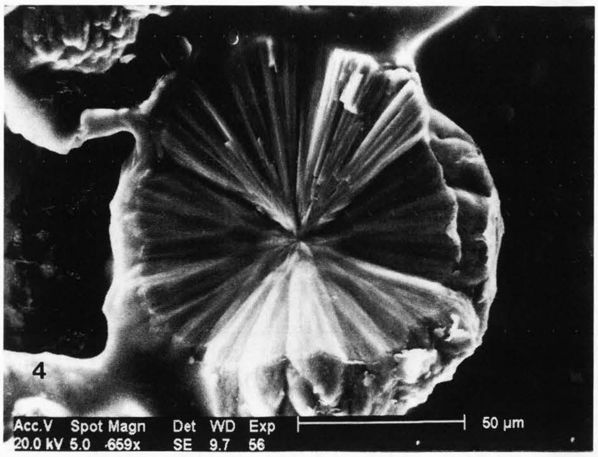
Table 2. Elemental analysis by EDX (giving data in weight percent[1]) of different areas of 16 sabulous samples.

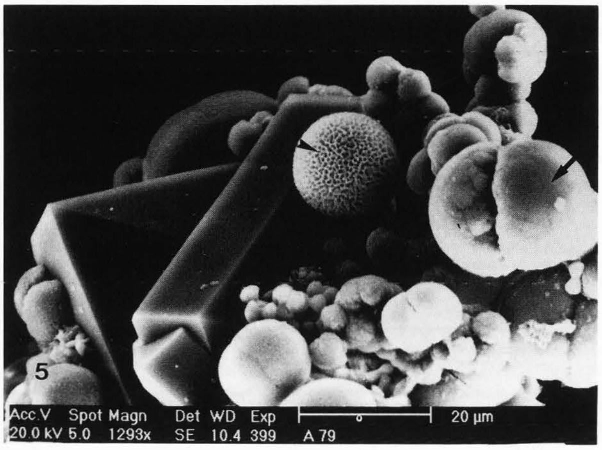
Sample	Ca	Mg	K	P	S	Si	Mn	Fe	Ni	Cu	Zn	Na	Cl
1	96.32	0.69		1.88		0.16	0.58	0.38					
	89.82	3.81		1.69	3.49	0.49	0.35	0.38					
	94.81	1.60		2.22		0.21	0.71	0.44					
2	94.36	3.87	0.71		1.06								
	91.42	3.88	2.76		1.94								
	90.98	1.65	2.59		1.73	0.28						2.09	0.64
3	82.75	5.97	1.72	2.25	2.97	1.30	2.21	0.85					
	88.37	1.82	1.09	4.30	3.97	0.27		0.38					
4	97.24	2.02			0.59		0.15						
	87.05	2.09	1.10	2.48	6.34	0.43	0.51						
	87.43	2.00	1.27	2.08	6.36	0.22	0.39	0.25					
5	90.00	5.86	0.64	1.75	0.97	0.60	0.19						
	90.40	4.64	0.58	2.00	1.26	0.57	0.28	0.28					
	92.66		0.83	2.49	1.71				1.14	0.34	0.83		
	90.93	2.12	0.79	2.86	1.76	0.58			0.48	0.30	0.18		
6	73.08	7.32	9.56	2.63	3.56	3.43			0.39				
	89.05	2.92	3.59	1.60	1.10	0.60		0.22	0.60			0.27	
	81.57	3.77	8.97	2.29	1.88	1.07			0.40				
	61.56	22.44	8.63	2.47	2.55	1.80			0.52				
	71.30	9.02	8.61	2.09	4.88	3.72			0.34				
7	84.95	5.39	2.16	6.07		1.44							
	73.27	9.31	5.63	9.15		2.64							
	77.72	5.66	4.87	10.14		1.61							
	80.36	3.74	1.82	11.64		2.44							
	66.22	1.50	4.00	27.72		0.55							
8	75.62	4.58	2.33	13.40	3.09		0.64	0.34					
	71.04	3.36	1.66	20.21	2.48	1.12		0.13					
	57.41	3.74	4.29	29.01	4.13	1.43							
9	64.92	2.83	2.03	28.26	0.69			0.19	0.64	0.19		0.19	
	67.50	2.22	2.07	27.59	0.60								
	83.96	3.13	1.15	11.20					0.36			0.18	
	80.92	4.63	1.62	10.65	1.38				0.38			0.38	
10	45.71	3.99	6.60	40.38								1.54	1.75
	42.84	4.26	7.69	41.43					0.15			1.48	2.13
11	58.02		3.64	32.61	5.31	0.42							
	74.57		2.93	21.12	1.38								
	55.50	0.28	3.82	34.91	3.30	1.08			0.68	0.17			0.22
12[2]	88.08	1.83	2.44	1.16	5.07	0.79							0.61
13	84.68	1.74	2.19	2.09	5.28	3.39			0.44				0.14
14	89.00	1.64	1.81	2.31	4.76	0.32	0.14						
	91.90	0.35	1.63	2.23	3.69	0.19							
	95.00	0.71	1.31	0.53	2.30		0.14						
15	87.51	2.66	4.50		5.32								
16	79.72		2.82	4.11	9.68	2.82	0.39	0.47					
	78.16	3.32	1.51	3.12	6.48	6.43	0.58	0.40					
	60.93	1.95	3.30	9.06	15.64	8.55	0.56						

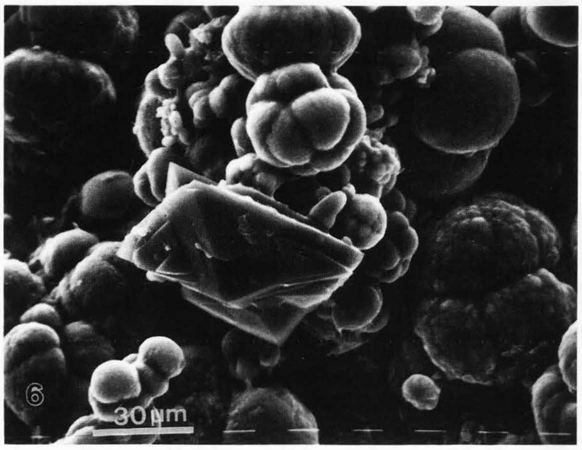
[1]Data on C, H, N and O not included (relative values given; the sum of detected elements has been normalized to 100%).

[2]Sample rich in organic matter.









Results and Discussion

The vesical samples were obtained: 62 from horses, 61 from donkeys and 17 from mules. The renal sample was of unknown origin. The specimens have been classified according to their mineral composition obtained by IR analysis. Calcium carbonate, in the crystalline forms of calcite and vaterite, was generally the predominant constituent. Other components found in small proportions were: calcium oxalates, apatitic calcium phosphates, calcium sulphate and silica.

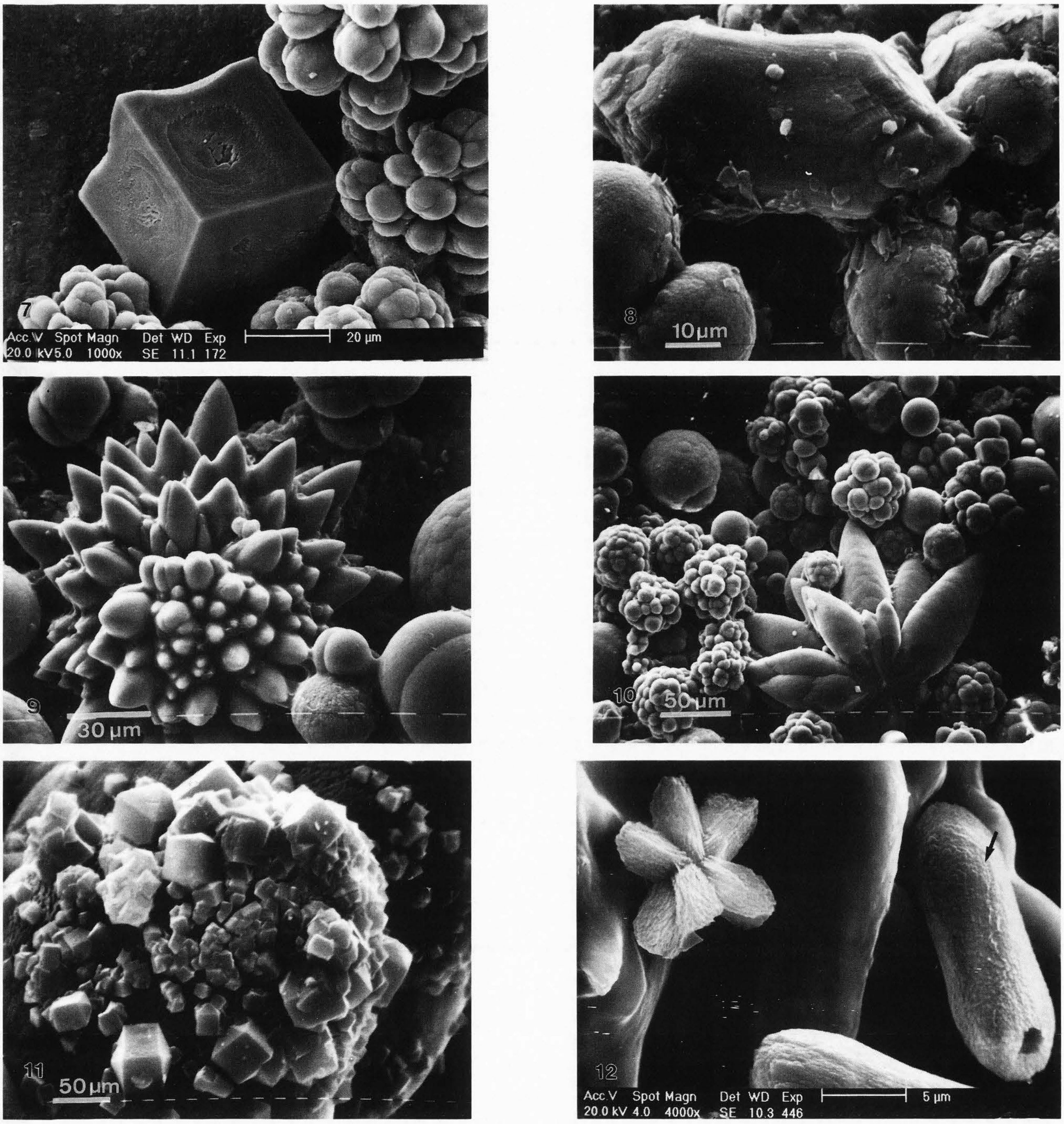
Figures 1 and 2 show examples of the IR spectra of two samples of sabulous material presenting: characteristic bands of calcite (1500-1400, 875, 712 cm^{-1}), vaterite (1500-1400, 875, 743 cm^{-1}), calcium sulphate (ca. 1150 cm^{-1}) and calcium phosphate (ca. 1040 cm^{-1}) in Figure 1; and calcite (ca. 1420, 875, 712 cm^{-1}), calcium oxalate as dihydrate and monohydrate forms (ca. 1640, 1320, 780, 520 cm^{-1}) and poorly crystalline calcium apatite (ca. 1040, 600, 560 cm^{-1}) in Figure 2.

Table 1 lists the qualitative component composition of the sabulous sediments analyzed by IR spectroscopy. Table 2 lists EDX elemental analysis results (semi-quantitative values) for 16 samples, excluding carbon, hydrogen, nitrogen and oxygen. The sum of the detected elements has been normalized to 100% and, therefore, the results indicate the relative weight percentages.

Several samples were also analyzed by X-ray powder diffraction (XRD). Calcite and vaterite could be easily detected, but the minority and probably amorphous components such as phosphates, sulphates and silica (and sometimes calcium oxalates when they were present in small proportion) could not be detected by the XRD method. Therefore, we have chosen IR spectroscopy for the analysis of the sabulous material.

Calcite and vaterite

Thirty-three samples (23.4%) were composed mainly of calcite (no other component was detected by IR spectroscopy). The most frequent morphology of calcite was spherulites of different sizes, most of them with an apparently smooth surface (Fig. 3). The fracture surfaces of calcite spherulites presented typical radial striations, generally radiating from a central core (Fig. 4). This radial structure has also been described for calcium oxalate monohydrate [2, 3, 16].



Figures 3-12 (Figs. 3-6 on the facing page, Figs. 7-12 above). Scanning electron micrographs: calcite sphere surrounded by smaller calcite spherulites [EDX: Ca:K:Mg = 89:3.6:2.9, plus other minority elements] (**Fig. 3**); a fractured calcite spherulite showing radial striations [EDX: Ca, Mg (traces), K (traces)] (**Fig. 4**); joined spherulites of calcite (arrow), a spherulite containing P (Ca:P = 88.4:4.3, arrowhead) and two bipyramids of weddellite can also be observed (**Fig. 5**); "spherular" and "pumpkin" shaped calcite crystals, a single weddellite crystal can also be observed in the middle (**Fig. 6**); calcite crystal with a rhombohedral morphology [EDX: Ca, Mg (traces)], spherular aggregates: probably vaterite (IR) (**Fig. 7**); laminar calcite: the rhombohedral growth of calcite can be observed in the borders of the crystal (**Fig. 8**); a vaterite "flower" surrounded by spherulites of vaterite (IR) [EDX: Ca, Mg (traces), K (traces)] (**Fig. 9**); a "flower" of vaterite, surrounded by "mulberries" of possible vaterite [EDX: Ca, Mg (traces)] (**Fig. 10**); calcium oxalate dihydrate crystals [EDX: Ca] (**Fig. 11**); and a crystal (arrow) with a "dumbbell" shape [EDX: Ca:Mg = 94.4:2.8; probably calcium oxalate monohydrate] (**Fig. 12**).

Sometimes spherulites appeared joined (Fig. 5); on other occasions, they had an irregular surface or were "pumpkin-shaped" (Fig. 6). Less frequently, calcite exhibited a rhombohedral morphology (Fig. 7), intermeshed with spherular aggregates. The rhombohedral growth of calcite could also be observed at the surfaces of a plate-shaped crystal of calcite (laminar calcite) (Fig. 8).

Seventeen sabulous deposits (12.1%) were composed mainly of vaterite. Vaterite crystals sometimes appeared in the form of "flowers" or "stars" (Figs. 9 and 10). On other occasions, vaterite showed the form of "mulberries" (Fig. 10). Other structures of vaterite which were occasionally seen were "smooth" and "irregular" surface spherulites (Figs. 7 and 9) similar to calcite (Figs. 3 and 6).

The EDX analysis of vaterite and calcite crystals revealed calcium as a major element. Magnesium and potassium were usually also present, but always in a much smaller proportion than calcium. Manganese, as well as other elements, were also found on some occasions but in a very low percentages (Table 2).

As shown in Table 1, the largest group of sabulous samples (29.8%) was composed of both calcite and vaterite. When calcite and vaterite appeared together, it was not possible to differentiate clearly between them by SEM when they showed a spherulitic morphology. In this case, the presence of both forms of calcium carbonate could be shown by the IR spectra.

The size of calcium carbonate crystals (calcite and/or vaterite) is variable. The medium dimension described in the literature is less than 0.1 μm [6]. In our study, most of the crystalline material was present in the form of aggregates, formed by small particles. Frequently, a certain amount of calcium carbonate took the form of fibrous radial-spherical aggregates (ooliths or microspheroliths), with a size between 10 and 150 μm.

As stated above, the most common composition found for equine sabulous deposits was a mixture of calcite and vaterite. Furthermore, it should be emphasized that in our samples, calcite and vaterite did not usually appear as "pure" compounds. In these two forms of calcium carbonate, calcium had been replaced by varying amounts of other elements, mainly magnesium and potassium, as confirmed by EDX analysis (Table 2). Grünberg [5] found an inverse ratio between the amount of magnesium content and the size of calcium carbonate crystals, so that there are higher magnesium concentrations in the smaller crystals.

In previous investigations, substituted vaterite has been found in both normal equine urine sediments and equine sabulous deposits [14, 15]. On the other hand, substituted calcite [5, 6] and substituted vaterite [14, 19] have also frequently been identified as components of equine discrete calculi. It has also been reported that calcium can be replaced by manganese in substituted vaterite [9, 14, 15, 19]. However, as stated above, in our study, manganese was only found in a few samples of both calcite and vaterite, although in a very small proportion.

Therefore, according to our results, we can conclude that "substituted calcite and vaterite" are the main constituents of equine sabulous deposits.

Calcium oxalates

Calcium oxalate dihydrate (weddellite) was frequently found in sabulous deposits, although generally in a minority proportion. However, calcium oxalate (mainly weddellite) accompanied calcium carbonate (calcite and/or vaterite) in 13% of the 141 sabulous deposits.

Weddellite crystals displayed their characteristic bipyramidal morphology [3, 11, 16, 21] (Fig. 6); sometimes, aggregates of bipyramids were also observed (Fig. 11). Calcium oxalate monohydrate (whewellite) appeared with less frequency than the dihydrate. Of the whewellite morphology, we only observed "dumbbell" or "hour glass" shaped crystals in our study (Fig. 12).

The stabilizing action of magnesium over weddellite crystals seems to inhibit their dehydration and transformation into calcium oxalate monohydrate, which is thermodynamically stable [3]. For this reason, we believe that the presence of significant quantities of magnesium in the equine urine, and consequently, in the sabulous deposits, could explain why calcium oxalate dihydrate is found much more frequently than the monohydrate in these deposits.

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Discussion with Reviewers

M. Daudon: The authors should provide more data in order to prove the identity of the crystals shown in Figure 12.

Authors: The identification of these crystals was not only based on the results obtained during the SEM/EDX analysis, but also on the IR spectra which were obtained from several portions of this specimen and which confirmed the presence of a small proportion of calcium oxalate, probably whewellite. Moreover, the infrared spectra excluded the presence of urates.

M. Daudon: What does "composed mainly of calcite" mean? In Table 1, these 33 samples are classified as pure calcite.

Authors: The specimens were classified according to their mineral composition obtained by IR analysis. However, as is well known, IR spectroscopy may be insensitive for detection of minor components in the sample. From the overall results, a purity of > 95% in the samples classified as pure calcite or pure vaterite could be estimated.

M. Daudon: What is the difference between "normal equine urine sediments" and "equine sabulous deposits?"

Authors: Normal equine urine sediments constitute a physiological finding in normal equine urine which is rich in calcium carbonate, calcium phosphates and calcium oxalates crystals. This significant proportion of crystals in normal equine urine provides its characteristic turbidity. These crystals can be identified by optical microscopy examination of the urine sediment. In contrast, equine sabulous deposits consist of the **pathological sedimentation** of sabulous (sandy) material in the equine urinary tract, mainly in the bladder, in the form of a semi-solid sedimented sludge. In the literature, the presence of huge quantities (up to 10 kg) of this material has been described [5]. Sabulous deposits are considered as a type of urolithiasis in equine species.

M. Daudon: Was the distribution of the various crystalline forms of calcium carbonate different when the animal species were considered separately?

Authors: Apparently, there was no different distribution of the various crystalline forms of calcium carbonate when the animal species were considered separately.

However, the incidence of sabulous material varied with the different species, and it represented 11.7% in donkeys, whereas in horses and mules, it was 5.8% and 3.9%, respectively.

M. Daudon: What is the explanation for the fact that aragonite, another crystalline form of calcium carbonate, was never detected?

Authors: We cannot explain why aragonite was never detected in the 141 samples studied. According to the literature, "in the absence of seed crystals and of bivalent cations other than Ca^{2+}, the $CaCO_3$ polymorph precipitating is most clearly determined by nucleation. The result that the polymorph forming at ordinary temperature is calcite, especially in slow precipitations, is in harmony with thermodynamic stability, whereas the formation of aragonite above about 30°C is not and must be explained by aragonite nucleation being favoured by increasing temperature and by greater growth rates of aragonite in comparison to calcite." On the other hand, "vaterite may nucleate and grow at ordinary temperature under suitable conditions. These are characterized largely by a sufficiently high ratio of CO_3^{2-}/Ca^{2+} in solution and correlate with the more open structure of vaterite" [25].

M. Daudon: Is the presence of urinary sabulous deposits a cause to discard the slaughtered animals and to decline their use as foodstuffs?

Authors: No. The presence of sabulous deposits is not a consideration in order to discard the slaughtered animals.

S. Deganello: Please comment on the usefulness of XRD work in this study. Identifications by SEM and IR alone can be erroneous. Those two techniques, in fact, have potentially limited discrimination when several phases and/or polymorphs are present, as is the case in this work.

Authors: For the present study, IR spectroscopy, SEM and EDX analysis techniques were selected. XRD measurements were carried out for several samples of sabulous material, but no additional information could be obtained. In fact, phosphates, sulphates and oxalates, when they were present in small proportion, were better detected by IR. Moreover, amorphous phosphate and silica could only be detected by the IR technique.

S. Deganello: It would be important to discriminate amongst the various calcium phosphates and sulphates which were found.

Authors: Due to the fact that these compounds generally appear in a very minor proportion, it was not possible to discriminate among the various possible types of phosphates and sulphates. In any case, this question will be the subject of a subsequent paper.

S.R. Khan: What is the nature of non-crystalline part of the deposits?

Authors: In normal equine urine, there is abundant organic material which is secreted by the mucus producing cells localized in the renal pelvis and in the urethral epithelium. Many authors state that this material (mainly mucoproteins) has an important role in gathering urine crystals. Moreover, amorphous phosphates and silica were also present in the material studied.

S.R. Khan: Urolithiasis is rare in equine species but sabulous deposits are common. Are sabulous deposits in the bladder not considered an aspect of urolithiasis?

Authors: Yes. Sabulous deposits are considered another form of presentation of urolithiasis in equine species: "sabulous urolithiasis."

M. Marković: How sensitive were your IR analyses? Did you find various compositions when different samples taken from the same urine deposit were analyzed?

Authors: Our IR analysis was sensitive for the compounds reported. In some cases, the results for phosphates and sulphates were confirmed by the IR analysis of calcinated samples. However, the presence of other minority constituents cannot be excluded. The infrared spectra of different portions of the same sample were rather similar. However, the EDX data showed that at a microscopic level, samples are heterogeneous, as can be deduced from Table 2.

M. Marković: Which calcium phosphate salts (i.e., amorphous calcium phosphates, brushite, apatites, octacalcium phosphate) were present in the mixture with calcium carbonates?

Authors: The calcium phosphate salts (generally, in a minor proportion) which were identified in this study were: amorphous calcium phosphate and carbonate apatite. Octacalcium phosphate, magnesium ammonium phosphate hexahydrate (struvite) and brushite were not detected in our samples.

M. Marković: Did you verify your results on substitution of calcium by magnesium and potassium in calcite and vaterite by XRD? Did you detect differences in diffraction line positions (d-values) for pure and substituted minerals? Was the presence of substituted calcite crystals in equine calculi and/or deposits previously reported?

Authors: The present study was limited to the use of IR, SEM and EDX techniques, although a few samples were also examined by XRD. Concerning the last part of your question, please see references [5, 6, 9, 14, 15, and 19].

M. Marković: Could you compare your results with previous data on equine calculi and normal equine urine deposits composition and indicate differences?
Authors: Previous data concerning normal equine urine deposits consider a much more reduced number of samples. This fact makes the comparison difficult. According to the literature, calcium carbonate is the predominant mineral component of equine urinary calculi, although there are isolated reports of other types such as oxalates and phosphates. Mair and Osborn [14] found that calcium carbonate in the crystalline form of calcite was the major component in each of 18 equine urinary calculi. Other commonly found components were weddellite and substituted vaterite.

In a study of 15 uroliths [24], we have found that the main constituent is calcite; other components found in smaller proportions were calcium phosphates, calcium oxalates and, in minor quantities, calcium sulphates. Vaterite was identified in two cases and amorphous silica, in one calculus.

Concerning the crystalline composition of normal equine urine deposits, Mair and Osborn [15] reported that calcium carbonate in the form of calcite was also the predominant crystalline component in the urine deposits. Substituted vaterite was present in 15 urine samples; weddellite in 12 samples and hydroxyapatite in 5 samples. Our results on sabulous deposits show, besides the mentioned constituents, the existence of substituted calcite and the presence of small quantities of sulphate. Silica was also found in one case.

Finally, several authors include struvite as a constituent of equine uroliths [4, 5, 12, 14]. We have not found this substance, either in sabulous deposits or in uroliths. This fact could be explained our having only studied slaughtered animals which were presumably free of urine infection. However, we have found struvite in equine enteroliths.

Additional References

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