TWINKLING ARTIFACT IN SMALL ANIMAL COLOR-DOPPLER SONOGRAPHY

ARNAUD LOUVET

Twinkling artifact is a recently described color-Doppler phenomenon that has not been studied in the veterinary field. Our purpose was to assess the grade of the artifact in vitro with varying urolith compositions, and to evaluate its potential role in clinical practice. Five canine and feline uroliths types of 100% mineral composition were studied in vitro with color-Doppler sonography, and a prospective study was performed in 41 patients with renal, bladder, gallbladder, or digestive focal hyperechogenicities. The images were analyzed for the presence and the grade of the artifact. Phantom study demonstrated the constant occurrence of the artifact regardless of the mineral composition of uroliths. Mottled and rough surfaced stones produced higher grades of twinkling artifact. High-grade, color-twinkling artifact generated by stones occured frequently in vivo. Bladder crystalluria was more frequently detected by artifact visualization than sample urinalysis performed by cystocentesis. In veterinary medicine, twinkling artifact may thus be considered an additional sonographic feature of urinary stones, and can lead to a more appropriate management of patients presenting gray-scale sonographic focal hyperechogenicities. *Veterinary Radiology & Ultrasound, Vol. 47, No. 4, 2006, pp 384–390.*

Key words: artifact, canine, Doppler studies, feline, ultrasound (US).

Introduction

COLOR-DOPPLER ULTRASOUND is increasingly being used in clinical practice; however, color-Doppler artifacts encountered during scanning of animals have not been extensively described. Artifactual color signals associated with hyperechoic stationary objects in the absence of flow were first described in 1996.[1] This artifact, called the twinkling artifact, occurs behind a strongly reflective interface, such as those produced by urinary tract stones or parenchymal calcifications, and appears as a quickly fluctuating mixture of Doppler signals with an associated characteristic spectrum of noise.[1] A narrow-band signal error generated by highly echogenic interfaces seems to be the primary cause of the artifact. Strong reflectors with rough surfaces will magnify this narrow-band signal to produce apparent aliased Doppler shifts.[2] The artifact appearance is also highly machine and setting dependent.[2]

As its first description, this artifact has been reported mainly in association with nephrolithiasis in humans.[3] A few studies link its occurrence with calcified or metallic structures.[4,5] Little is known about the twinkling artifact in relation to canine and feline uroliths. Our purpose was (a) to determine in vitro whether the presence and the grade of the twinkling artifact are related to the morphology and biochemical composition of canine and feline uroliths, and (b) to describe several instances of the twinkling artifact generated in vivo by different mineralized structures.

Materials and Methods

In Vitro Study

Canine or feline bladder uroliths of 100% mineral composition by optical crystallography and infrared spectroscopy were chosen. The five mineral types were magnesium ammonium phosphate hexahydrate, calcium oxalate monohydrate, calcium oxalate dihydrate, cystine, and ammonium acid urate. Surface characterization of all stones was performed visually and classified as smooth, mottled or crystalline according to previously established criteria.[6] Each specimen was placed between two disposable aqueous standoff pads* for imaging. All sonographic examinations were performed by one operator using a 3–12 MHz linear probe.† All uroliths were studied with identical imaging conditions. The gray-scale images were acquired with three focal zones placed immediately below the surface of the uroliths. The transducer frequency, 2D gain, and mechanical index (MI) output power were set at 12 MHz, 65%, and 1.4% (maximum value), respectively. The color-Doppler images were acquired using fundamental imaging (without harmonics), with the Doppler frequency set at 3.8 MHz and using the lowest wall filter. Color-write priority was set to the maximum value. Each urolith was

From the Small Animal Veterinary Clinic, 80 rue Péreire, 78100 Saint Germain-en-laye, France.

Address correspondence and reprint requests to Arnaud Louvet, at the above address. E-mail: ladelou@wanadoo.fr

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*Aquaflex, Parker Laboratories Inc, Fairfield, NJ.

†EnVisor; Philips, Bothell, WA.

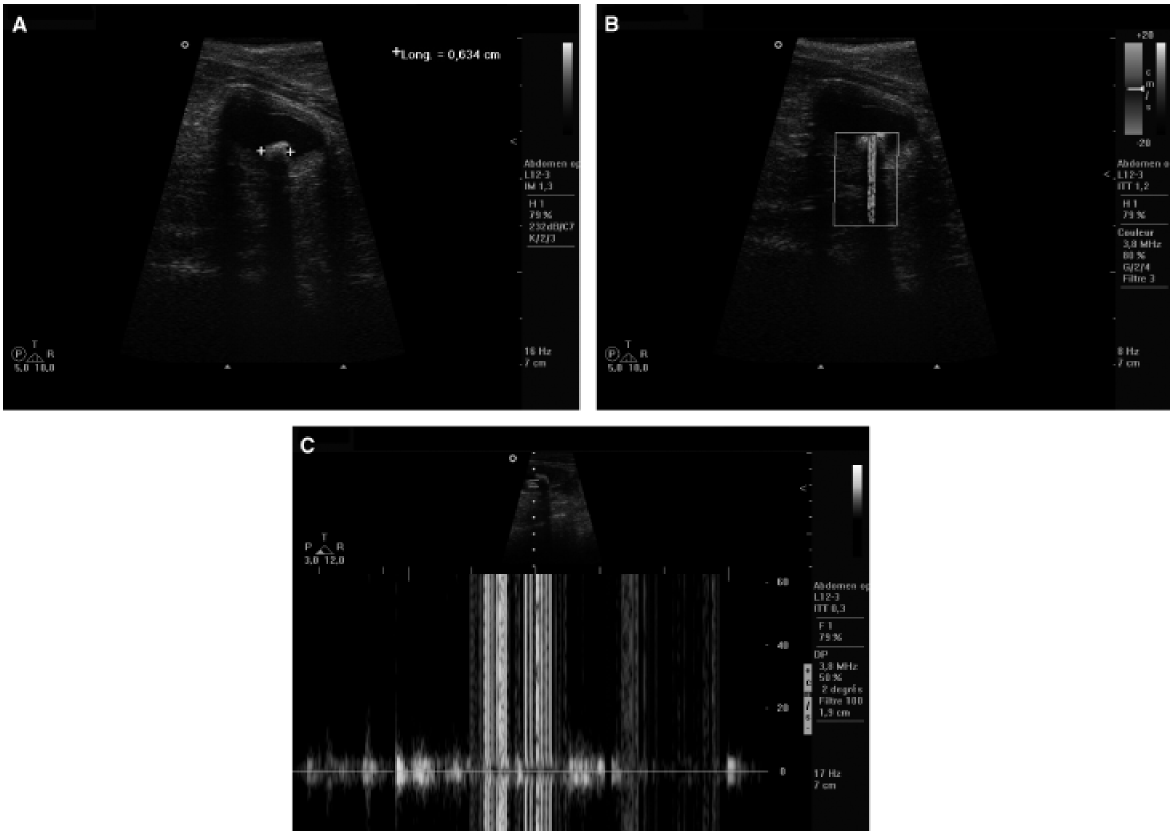

FIG. 1. Calcium oxalate monohydrate and dihydrate (70% and 30%, respectively) stone in a cat. (A) In the gray-scale sagittal image, there is a focal hyperechogenicity that shadows in the dependent portion of the bladder. (B) With color-Doppler, there is a grade 3 twinkling artifact behind this urolith. (c) Pulsed-wave Doppler spectrum of a calcium oxalate stone in a dog. Note the broadband signal in the middle part of the spectrum typical of the twinkling artifact and the narrow-band signal surrounding the baseline because of the movement of the animal and/or the transducer.

successively imaged with Doppler gain 60% and 75%. The twinkling artifact was graded 0 when absent, 1 when present but occupying only a small portion (height <5 mm) of the color box below the reflective interface, 2 when occupying a larger portion of the color box ($0.5\,cm < height < 2\,cm$), and 3 when largely overflowing the reflective interface (height >2 cm).

In Vivo Study

Gray-scale, color-Doppler, and spectral Doppler sonography were performed prospectively in patients between May and October 2005.

All sonographic examinations were performed by one operator using a 3–12 MHz linear probe and/or a 5–12 MHz sectorial-phased array probe.† For visualization of posterior acoustic shadowing, focal zones were always placed at the depth of or slightly deeper than the stone. In color-Doppler sonography, a red-and-blue color map was used, and the color window size was adjusted to cover the echogenic focus and adjacent tissue. The color-Doppler gain was set just below the threshold for color noise.[1] The presence of a color signal in the hyperechoic area was assessed relative to adjacent soft tissue. Doppler spectra composed of close vertical bands with no outer wrapping were sought at the echogenic interface, not within the color artifact limits (Fig. 1). The artifact-grading scheme was identical to that used in vitro. Stones were differentiated from wall calcifications if movement to the dependent portion of the bladder or gallbladder could be identified. The term renal stone/calcification was used when differentiation could not be obtained. The term crystalluria was used to characterize sediment composed of an aggregate of microscopic crystals, whereas the term stone was used to describe a macroscopic calculus.

Data Analysis

Groups were compared using Student's *t*-test for parametric data. A probability value of $P<0.05$ was considered significant.

†EnVisor; Philips, Bothell, WA.

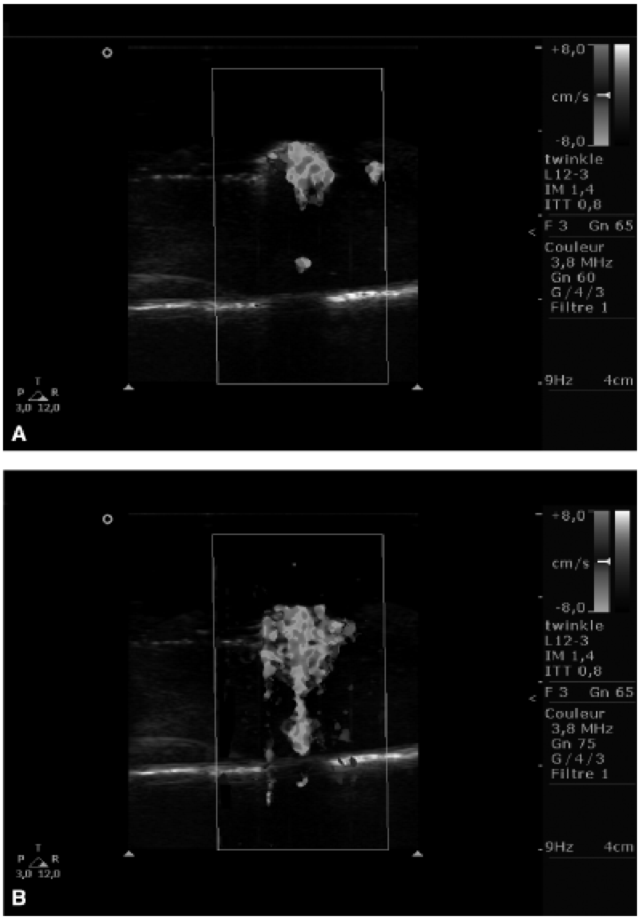

FIG. 2. Influence of Doppler gain on twinkling artifact grade. (A) Color-Doppler image of a struvite stone with 60% gain with a grade 2 artifact. (B) Color-Doppler image acquired with a 75% gain with a grade 3 twinkling artifact.

Results

In Vitro Study

The twinkling artifact was observed consistently behind all uroliths, regardless of their mineral composition, size, and surface or of gain settings.

Twinkling artifact was dependent on gain settings, with higher grades obtained for higher color-Doppler gains (Fig. 2, Table 1): artifact grade increased consistently for calcium oxalate, urate, and cystine uroliths when Doppler gain was set at 75% compared with 60%; however, color-column length did not increase sufficiently for struvite stones to change the artifact grade, despite increased Doppler gain settings.

Twinkling grade was not statistically different between the five mineral compositions.

Twinkling artifact was dependent on stone size, with larger stones producing higher artifact grades (Table 2).

Stone surface was shown to influence artifact grade (Table 3, Fig. 3). Regardless of color-Doppler gain settings, mottled surfaced stones produced the strongest artifact grade. Smooth surfaced stones produced the lowest artifact grade when the color-Doppler gain was 60%. For higher color-Doppler gain (75%), a strong and moderate increase in twinkling artifact grade was observed for mottled and smooth surfaced stones, respectively, whereas artifact grade was unchanged for crystalline-surfaced stones.

In Vivo Study

In all, 41 patients were enrolled during the study period. The circumstances under which a twinkling artifact was observed were the following: renal stone/calcification 19.5% (8/41 patients) (Fig. 4), bladder stones 19.5% (8/41 patients), bladder crystalluria 56% (23/41 patients), gallbladder stones 2.5% (1/41 patients), and stomach mineralized foreign body 2.5% (1/41 patients). Among all calculi detected by gray-scale sonography, 87.5% were subsequently analyzed. 85.7% of these were predominantly composed of calcium oxalate and 14.3% were of struvite composition. Among all instances of crystalluria, only 52% were subsequently analyzed. 66.6% of these were of struvite composition; 25% and 8.4% were predominantly composed of calcium oxalate and urate, respectively (Table 4).

All focal bladder hyperechogenicities were found to generate a twinkling artifact. Bladder uroliths were found to generate higher artifact grades than bladder crystals. Although this finding was significant for calcium oxalate specimens ($P = 0.02$), the number of patients examined was too low to allow a general statistical comparison. Calcium oxalate stones were frequently composed of monohydrate and dihydrate moieties in variable proportions. The twinkling artifact grade was always 2 or higher (mean 2.8).

Among all instances of crystalluria, grade 2 was highly predominant (83%). Grade 3 artifact was not encountered with crystalluria.

TABLE 1. Twinkling Artifact Grade According to Stone Mineral Composition and Gain Setting

	CaOx1 ($n = 3$)	CaOx2 ($n = 4$)	Urate ($n = 1$)	Struvite ($n = 8$)	Cystine ($n = 3$)	Total ($n = 19$)
Gain 60%	2^*	1.75 ± 0.5	1	2 ± 0.53	2.67 ± 0.58	2 ± 0.58†
Gain 75%	3	2.25 ± 0.5	2	2 ± 0.53	3	2.37 ± 0.60

Data are expressed as mean ± standard deviation. $^*P = 0.001$, significantly different versus the corresponding value in the Gain 75% group (Student's *t*-test). †$P = 0.061$ using the *t*-test.CaOx1, calcium oxalate monohydrate; CaOx2, calcium oxalate dihydrate.

TABLE 2. Twinkling Artifact Grade According to the Stone Size for the Same Gain Settings (60%)

Size	Twinkling Grade
< 5 mm ($n = 4$)	1.5 ± 0.58*
6–10 mm ($n = 12$)	1.9 ± 0.29*
> 10 mm ($n = 3$)	3 ± 0

Data are expressed as mean ± standard deviation. *Significantly different vs. the corresponding value in the > 10 mm group ($P < 0.05$).

Artifact grade was higher when the gray-scale amount of the sediment increased (Fig. 5). In more than half of the instances of grade 1 twinkling artifact caused by suspected crystalluria, subsequent urinalysis failed to demonstrate any abnormal findings. Renewal of urinalysis was necessary to confirm the presence of crystals.

Discussion

Urinary or gallbladder stones can easily be detected sonographically when they have both distinct echogenicity and discrete posterior acoustic shadowing. However, in clinical practice, we encounter many equivoqual situations in which it is difficult to determine whether a stone is present because of indistinct echogenicity, faint or absent posterior acoustic shadowing, and/or an empty bladder or gallbladder. Indistinc echogenicity because of surrounding sonic-beam attenuating fat is a well-known situation in the human renal sinus.[3] Acoustic shadowing is considered the cornerstone of urolith identification in the urinary tract. However, shadowing occurred relatively infrequently (e.g., less than 25% of the time for most canine pure mineral uroliths regardless of mineral type) in an in vitro study.[7] In human sonography, the twinkling artifact is frequently, although unconsistently, observed behind calcifications. In anecdotal reports, the twinkling artifact has also proved useful for the detection of encrusted (calcified) indwelling ureteral stents[8]; a retro-orbital metalic foreign body, calcifications within a mass of phtisis bulbi or optic disk calcifications;[4] and a neurosurgical coil within a cerebral aneurysm.[5] The twinkling artifact is generated by a random, strongly reflecting medium with a rough interface composed of individual reflectors.[1,2] In most instances, it appears when the surface of calculus is irregular. In vitro, no twinkling artifact was generated with a flat interface of smooth steel or brass wire.[2]

TABLE 3. Twinkling Artifact Grade According to the Stone Surface with Varying Gain Settings

	Smooth ($n = 9$)	Mottled ($n = 5$)	Crystalline ($n = 5$)
Gain 60%	1.89 ± 0.33*	2.2 ± 0.6	2.0 ± 0.71
Gain 75%	2.33 ± 0.50	2.8 ± 0.45	2.0 ± 0.71

Data are expressed as mean ± standard deviation. *$P = 0.041$, significantly different vs. the corresponding value in the Gain 75% group.

The relationship between the biochemical composition of urinary stones and the grade of twinkling artifact has been studied in vitro.[2,3,6] Calculi of calcium phosphate, calcium oxalate dihydrate,[6] and struvite[2] always produced this artifact. Regardless of their mineral composition, the twinkling artifact was observed consistently behind stones of less than 5 mm or more than 10 mm diameter.[3] Absence of the twinkling artifact was noted in 42% and 40% of patients with calcium oxalate monohydrate and urate stones, respectively.[6] In this latter study, absence of the twinkling artifact was considered predictive of calcium oxalate monohydrate stone with a sensitivity and specificity of 60% and 83%, respectively. In contrast to this study, but in accordance with a phantom study,[3] all uroliths examined in our study produced a twinkling artifact, including calcium oxalate monohydrate and urate stones. In our in vitro study, the mean twinkling artifact grade for calcium oxalate monohydrate was two. In vivo, calcium oxalate uroliths were frequently composed of mono and dihydrate moieties in a variable percentage and the mean twinkling grade was 2.8. The only one 100% monohydrate composition had a grade 2 twinkling artifact. Stones and crystals of urate composition were insufficient in number to draw any conclusions. Discrepancies between other studies and our own are probably related to our lower number of stones, and differences in equipment and machine settings; however, we confirm that the twinkling artifact is a frequent phenomenon for renal and bladder stones.[3]

Overall, for set machine parameters, there was a trend for mottled and larger surfaced uroliths to produce higher grades of the twinkling artifact, in agreement with its first description.[1] More recently, a comparison of artificial surfaces of progressively increasing roughness has also confirmed that increasing roughness broadens the Doppler spectrum.[2] In our in vitro study, all smooth-surfaced uroliths generated a twinkling artifact (mean 1.89 for 60% color-Doppler gain). This contradicts findings from a previous study in which smooth stones did not produce an artifact.[6] Indeed, in our study, the twinkling artifact was even found for smooth stones of less than 4 mm diameter. In accordance with previous findings,[6] for a 60% color-Doppler gain, mottled and crystalline stones produced higher artifact grades compared with smooth stones. When Doppler gain was shifted from 60% to 75% in vitro, a trend toward an increase in artifact grade was observed for both smooth and mottled stones. The same phenomenon was observed for crystalline stones although this was insufficient to increase the grade score. Lowering the criterion threshold for grade 3 to 1.5 cm would likely have changed the results of our study. Twinkling artifact grade was shown to be related to stone size in our in vitro study (Table 2). In vivo, the number of stones examined was too low to allow a statistical comparison; however, the potential same finding as for the in vitro study seems intuitive. In

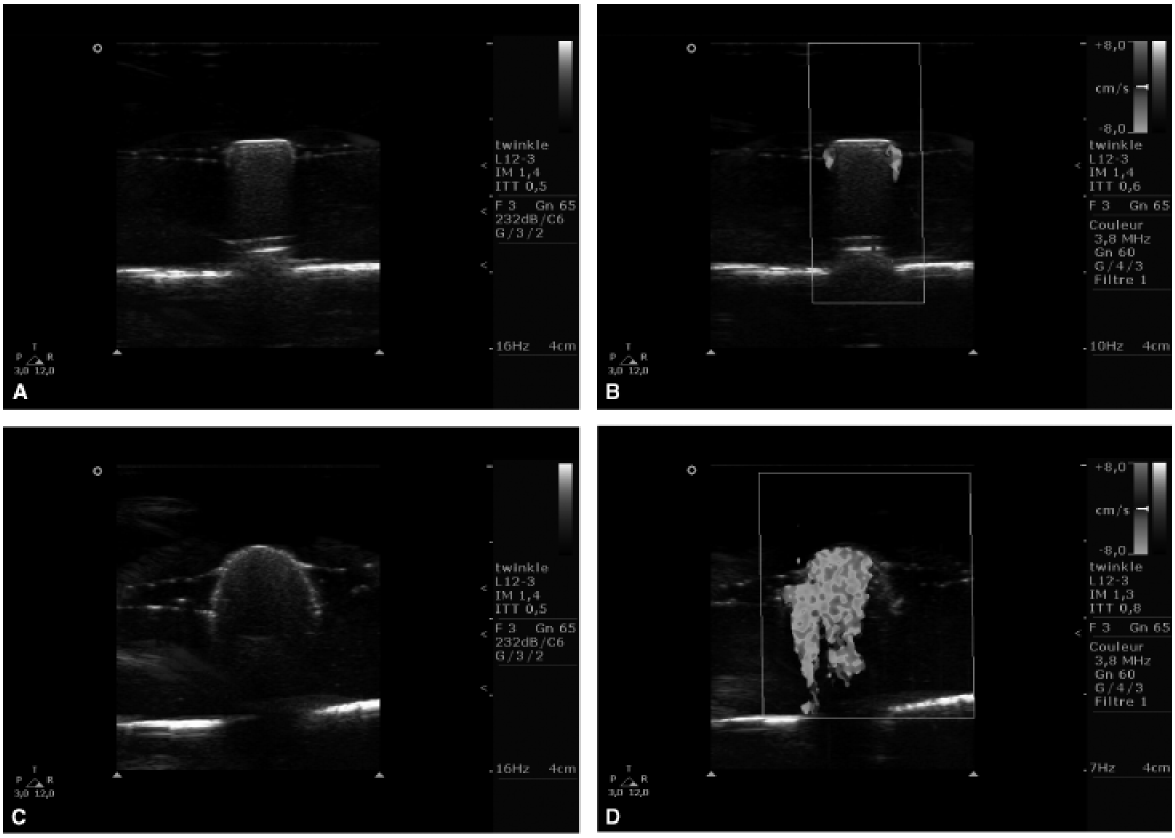

FIG. 3. Influence of urolith roughness on twinkling artifact. (A) Gray-scale in vitro ultrasound image of a smooth-surfaced cystine urolith. (B) Apllied color-Doppler. There is a grade 1 twinkling artifact emanating from lateral margins of the stone. (C–D) Gray-scale and color Doppler image of a rough-surfaced cystine urolith. There is a grade 3 twinkling artifact.

another study, the twinkling artifact was present behind 75% of stones for which the diameter was comprised between 6 and 10 mm and 100% of stones measuring more than 10 mm in diameter.[3] However, in the same study, twinkling artifact was also observed behind 100% of stones of less than 5 mm in diameter. To synthesize our and previous studies, twinkling artifact should be detected regardless of stone size, surface, or mineral composition but higher artifact grades are expected for rougher and larger stones.

Twinkling artifact has been shown to be ultrasound (US) system and machine settings dependent. In one study, the twinkling artifact was found in association with only 39% of urinary tract stones when an old generation system using analog technology was used, but was present in 96% of patients when a new generation system with digital processing technology was used.[9] An experimental investigation of the twinkling artifact and its appearance with various machine settings has recently been published.[2] In this study, the appearance of the artifact was highly dependent on the gray-scale gain and color-write priority settings. However, some effects were nonlinear and sometimes unexpected. In another study, artifact intensity was minimally affected by gain settings but strongly affected by the acoustic power.[9] Given the complexity of the role of interrelated machine settings in the appearance of the artifact, we chose to use a unique machine configuration for the entire in vitro study. A fixed maximal MI was used to favor artifact occurrence and color-Doppler gain was the only varying parameter. We found a definite relationship between artifact intensity and color-Doppler gain (Tables 1 and 3).

In vivo, our study is in agreement with human reports that describe the frequent association between twinkling artifact and urinary stones.[1] The artifact has been described as potentially useful in clinical practice for confirming the presence of a urolith, especially when technical conditions are difficult. Particular utility of the artifact has been described in the scenario of indistinct echo difference between the stone and adjacent parenchyma, and with faint or absent posterior acoustic shadowing. We found this artifact highly useful for the detection of bladder crystalluria. Although visualization of crystals is easy in a distended bladder, it is not unusual to encounter a collapsed bladder in patients with inflammatory diseases of the lower urinary tract. In this situation, gray-scale sonography often reveals a hyperechoic sediment lodged between the mucosal folds of the bladder, and acoustic shadowing is inconstant.[10] Blood clots have been described to produce a similar image. Twinkling artifact was found in all instances of blad-

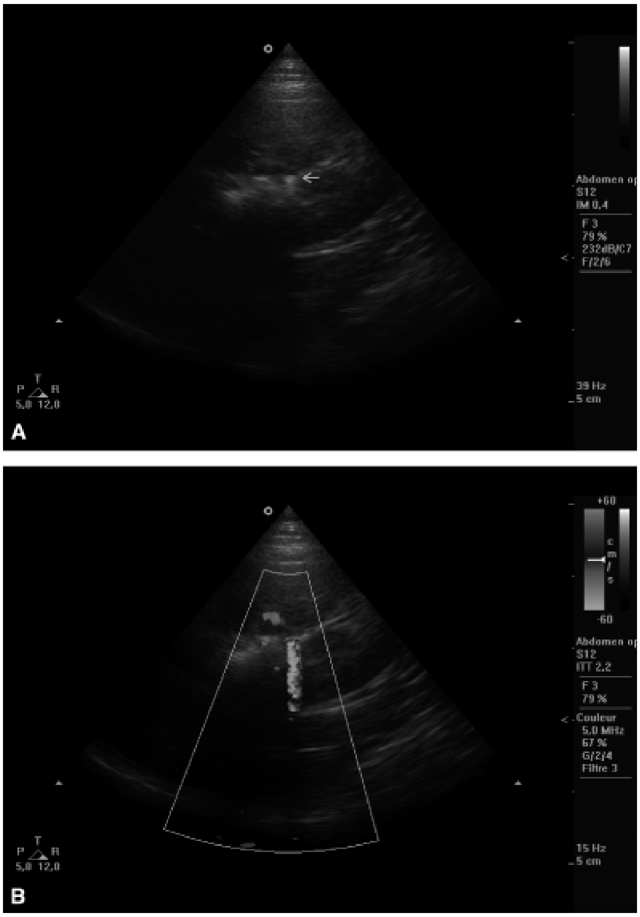

FIG. 4. Twinkling artifact identifies a kidney stone. (A) Gray-scale image of a canine kidney. There is a hyperechoic linear interface with a faint shadowing in the caudal pole of the sinus (arrow). (B) color-Doppler. There is a grade 2 twinkling artifact behind the caudal part of the sinus and normal color display in the renal vessels above the sinus. Without this artifact, the stone is difficult to diagnose.

der crystalluria in the dog and cat, regardless of the crystal's mineral composition (Table 4). Moreover, the twinkling artifact grade was frequently appreciated as significant (i.e., more than grade 1). The sediment was thought to act as an aggregate of microscopic reflective interfaces, thus generating a strong color twinkling artifact. Interestingly, we were not able to find any twinkling artifact when blood clots were imaged during our in vitro study (data not shown). In many instances of grade 1 twinkling artifact caused by suspected crystalluria, subsequent urinalysis failed to demonstrate any abnormal findings. Renewal of urinalysis was necessary to confirm the presence of crystals. In these situations, twinkling artifact enabled direct visualization of crystals located in folded portions of the bladder wall that urine sampling was unable to detect. Color-Doppler US was therefore judged to be more effective than urinalysis in the detection of low-grade crystalluria.

It has been hypothesized that urolith motion could influence the appearance of the artifact. Most experimental in vitro studies have thus used a clamped transducer and sometimes embedded uroliths in a modeling adhesive to avoid motion of the target. These procedures are necessary to ensure that artifact signal intensity is only affected by one of the tested machine settings. Our in vitro study was designed to assess, under conditions as close as possible to in vivo situations, the presence and subjective grade of the twinkling artifact, and subtle urolith motion was therefore not considered an obstacle in our image interpretation.

Our study was limited in vitro by the small number of uroliths examined. It was only possible to analyze between one and eight stones for each mineral composition and silicate stones were not included in our study. Our preliminary in vivo results are insufficient to support the role of color-Doppler sonography in the noninvasive determination of urolith composition. Further studies are needed to address this issue. Finally, our study did not consider the complex physical mechanisms responsible for the generation of the twinkling artifact. A recent publication has dealt with this matter.[2]

Conclusion

As has been done in humans, we confirm the role of color-Doppler US in highlighting the presence of uroliths in animals. In particular, the twinkling artifact adds important information in situations when nonspecific hyperechoic sediment is present. In the clinical setting, stone size and roughness seem to be the predominant calculus factors re-

TABLE 4. Summary of Mineral Composition, Twinkling Artifact Grade, and Stone Size Evaluated In Vivo in Dogs and Cats

	Number of Observations	Number of Stones Analyzed	Mineral Composition	Twinkling Grade (Mean ± SD)	Stone Size (mm)
Kidney calcification/stone	8	0	NA	2.14 ± 0.69	3.29 ± 0.91
Bladder stone	8	7 (87.5%)	CaOx, $n=6$ (85.7%)	2.8 ± 0.45	6.21 ± 2.88
			Struvite, $n=1$ (14.3%)	3	18
Bladder crystalluria	23	12 (52%)	CaOx, $n=3$ (25%)	1.67 ± 0.58	NA
			Struvite, $n=8$ (66.6%)	1.86 ± 0.38	NA
			Urate $n=1$ (8.4%)	2	NA
Gallbladder stone	1	0	NA	3	2.2
Bowel stone	1	0	NA	3	20

NA, not available; CaOx, calcium oxalate.

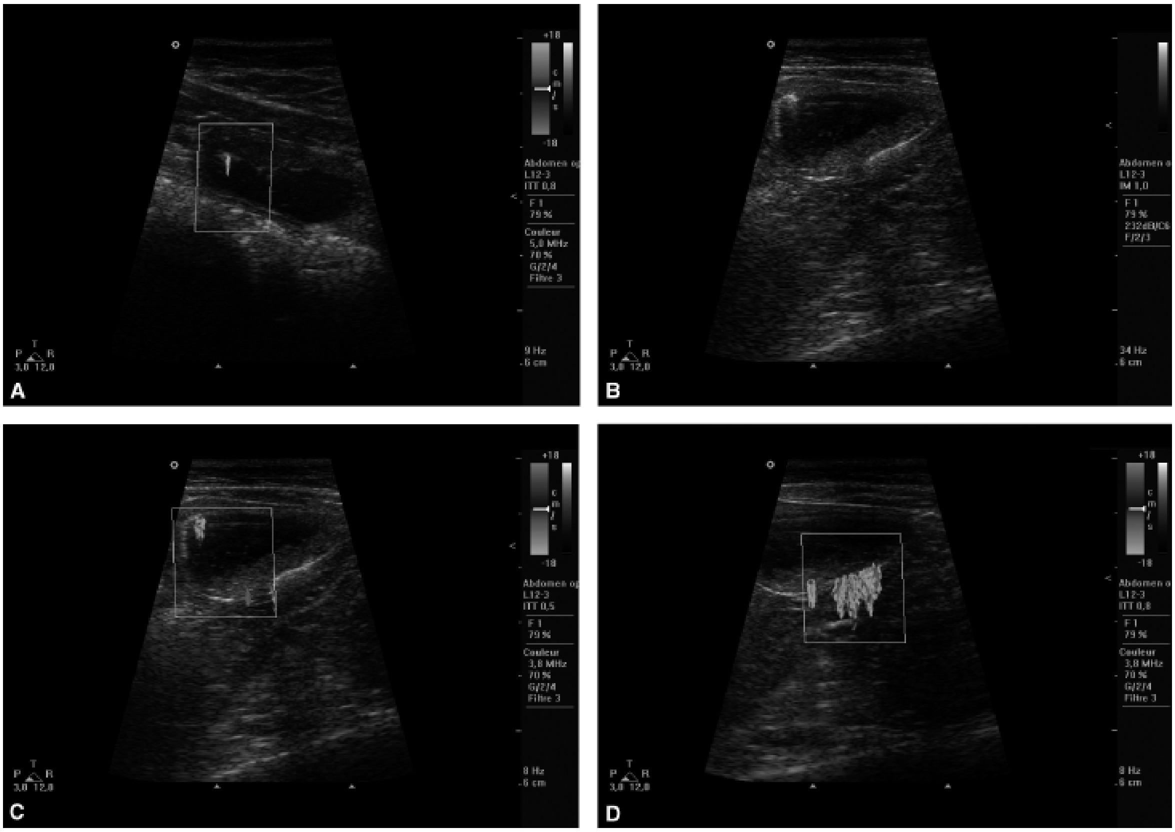

FIG. 5. Twinkling artifact behind struvite crystalluria in the cat. (A) Sagittal color-Doppler image of the bladder. There is a grade 1 twinkling artifact. (B) Sagittal gray-scale image of the bladder. There are nonspecific hyperechogenicities lodged between the cranial folds of the mucosa. (C) Color-Doppler image. There is a grade 2 twinkling artifact behind these hyperechogenicities. (D). Color-Doppler image of a patient with struvite crystalluria. There is a grade 3 twinkling artifact.

sponsible for the grade of the twinkling artifact. Twinkling artifact grade is also color-Doppler gain dependent. We also described the interesting role of this artifact in the detection of bladder crystalluria. Finding a twinkling artifact allows for the strong suspicion of a mineralized and rough-surfaced material. As this artifact appears to be dependent upon the technology used and newer generation machines are becoming available in veterinary medicine, it is anticipated that the more frequent detection of this artifact will progressively help us to define its role in veterinary US.

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