Mineral composition of urinary calculi from potbellied pigs with urolithiasis: 50 cases (1982–2012)

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**Objective**—To determine the mineral composition and anatomic location of urinary calculi and to investigate sex and reproductive status as predisposing factors for development of urolithiasis in potbellied pigs.

**Design**—Retrospective case series

**Samples**—Urinary calculi from 50 purebred and crossbred potbellied pigs.

**Procedures**—Laboratory records for urinary calculi of potbellied pigs submitted to the University of California-Davis Stone Laboratory from 1982 through 2012 were reviewed. Mineral composition of calculi was determined by polarized light microscopy, infrared spectroscopy, and, in some cases, x-ray diffractometry.

**Results**—Of the 48 urinary calculi analyzed by infrared spectroscopy, 21 (44%) were composed primarily of amorphous magnesium calcium phosphate; another 9 (19%) were primarily composed of calcium phosphate in the form of apatite. Of 50 urinary calculi, 22 (44%), 14 (28%), 10 (20%), 3 (6%), and 1 (2%) were removed from the urinary bladder only, urethra, both urinary bladder and urethra, urine, and renal pelvis, respectively. Sex of 6 potbellied pigs was not recorded. For 44 urinary calculi, 41 (93%) were from males (11 sexually intact males and 30 castrated) and 3 (7%) were from females (2 sexually intact females and 1 spayed). Among males, 73% (30/41) of submissions were from castrated males.

**Conclusions and Clinical Relevance**—In contrast to results from studies in commercial pigs, the most common composition of urinary calculi identified in purebred and crossbred potbellied pigs was amorphous magnesium calcium phosphate. Potential predisposing factors for urolithiasis in potbellied pigs may be similar to those for urolithiasis in commercial pigs. These include diet, urinary tract infections, and sex. Thus, prevention of urolithiasis should target these potential predisposing factors. (J Am Vet Med Assoc 2013;243:389–393)
become available over time to aid in calculi identification. The objectives of the study reported here were to determine the mineral composition of urinary calculi removed from potbellied pigs with not only polarized light microscopy but also infrared spectroscopy and, in some cases, XRD. Also the anatomic location of urinary calculi and the sex and reproductive status of affected potbellied pigs were investigated as possible predisposing factors for development of urolithiasis.

Materials and Methods

Case selection—Laboratory records of urinary calculi from all potbellied pigs that were submitted for analysis to the Gerald V. Ling Urinary Stone Analysis Laboratory at University of California-Davis from 1982 through 2012 were reviewed. Urinary calculi of potbellied pigs had been submitted from the William Pritchard Veterinary Teaching Hospital at the University of California-Davis, other veterinary medical teaching institutions, and private veterinary medical practices within the United States.

Laboratory records reviewed—Data abstracted from completed questionnaires that accompanied the submitted urinary calculi included the following: breed, sex, age, reproductive status, and the anatomic location of calculi at the time of surgical or nonsurgical removal. Results of bacteriologic cultures of urine samples and urinary calculi, if performed, were also reviewed.

Analysis of urinary calculi—Distinct layers of each urinary calculus were evaluated by the oil immersion method of optical crystallography via polarized light microscopy, as previously described. The 5 layer categories that were evaluated were as follows: the outer layer, layer 1, layer 2, layer 3, and the core layer. Layer composition was reported as percentage composition (eg, outer layer; 99% apatite and 1% struvite). Identification of urinary calculi in terms of type was made on the basis of the mineral with the greatest proportion (>70%).

Following assessment by polarized light microscopy, urinary calculi were also evaluated with a Fourier-transformed infrared spectrometer equipped with spectroscopy software and kidney stone library and analysis software, as previously described. Urinary calculi were then grouped for this study according to the predominant mineral. Powder XRD analysis was undertaken as previously reported for selected urinary calculi from each mineral group (ie, AMCP, apatite, struvite, calcium oxalate, or calcium carbonate) as well as for 3 calculi that were not classified into any mineral group to confirm the results of microscopic and infrared spectroscopy analyses.

Bacteriologic culture of urinary calculi—Preparation of urinary calculi for aerobic bacteriologic culture was performed as previously reported. Briefly, each calculus was washed 4 times with 50 mL of a sterile saline (0.9% NaCl) solution. A volume of 0.1 mL from the first and fourth wash was streaked on defibrinated 5% sheep agar plate with a sterile loop. Following the fourth wash, each calculus was rinsed again with sterile saline solution. Each calculus was then cracked with a Rongeur forceps, followed by removal of the center of the calculus with a sterile dental explorer. The center of the calculus removed was then crushed in a sterile mortar and pestle and mixed with 1 mL of sterile saline solution. A volume of 0.1 mL of the resulting slurry was then streaked on defibrinated 5% sheep agar plate with a sterile loop. Inoculated agar plates were incubated at 37°C and monitored for bacterial growth.

Statistical analyses—Because of the nonnormal distribution for age, descriptive statistics for this variable were reported as median (range) values. Number (percentage) of urinary calculi from potbellied pigs were determined and reported on the basis of sex (male or female), reproductive status (sexually intact or neutered), and mineral composition. A 2 test was performed to determine whether sex (male or female) and reproductive status (sexually intact vs spayed or castrated) increased the frequency of calculi submission. In cases where a cell had < 5 counts in the first frequency table, a Fisher exact test was performed. All analyses were performed with commercially available software. Values of P < 0.05 were considered significant.

Results

Information from laboratory records of 50 urinary calculi submitted from 50 potbellied pigs was included in the study. Urinary calculi originated from potbellied pigs in California, Georgia, North Carolina, Ohio, and Texas.

Sex of 6 potbellied pigs was not recorded. Of 44 urinary calculi, 41 (93%) were from males (11 sexually intact and 30 castrated) and 3 (7%) were from females (2 sexually intact and 1 spayed). Among males, 73% (30/41) of urinary calculi were from castrated males. A significantly (P = 0.029) higher number of urinary calculi were submitted from male potbellied pigs, compared with female potbellied pigs. Among males, there was a significantly (P = 0.001) higher number of urinary calculi submitted from castrated males, compared with sexually intact males. Median (range) age of potbellied pigs was 4 years (1 to 14 years).

Of 50 urinary calculi, 22 (44%), 14 (28%), 10 (20%), 3 (6%), and 1 (2%) were removed from the urinary bladder only, urethra, both urinary bladder and urethra, urine, and renal pelvis, respectively. Urinary calculi were significantly (P = 0.041) more often removed from the urinary bladder, compared with other sites in the urinary tract. Bacteriologic cultures were performed for 5 of 50 (10%) calculi, and no bacteriologic growth was observed.

Optical crystallography with polarized light microscopy revealed that the majority (29/50 [58%]) of urinary calculi contained primarily apatite. In 30 of 50 (60%) urinary calculi, there were ≥ 2 mineral types in the layers analyzed. Following assessment by polarized light microscopy, adequate amounts of sample material were available from 48 of 50 urinary calculi for evaluation with infrared spectroscopy. Urinary calculi were categorized on the basis of primary mineral composition as determined by infrared spectroscopy. Of the 48 urinary calculi, 21 (44%) were composed of AMCP, 9 (19%) were composed of apatite, 7 (15%)
were composed of struvite, and 4 each (8%) were composed of calcium oxalate or calcium carbonate. Of the 3 remaining urinary calculi, 1 contained newberyite, 1 contained bilirubin, and 1 had a complicated infrared spectroscopic pattern for which no accurate identification could be made.

Representative infrared spectroscopic (Figure 1) and XRD (Figure 2) patterns for urinary calculi containing primarily AMCP and apatite are shown. The infrared spectroscopic pattern for AMCP had broad peaks at 900 to 1,200 cm\(^{-1}\) (primary peak) with a shoulder at the lower wave number side and at 500 to 700 cm\(^{-1}\) (secondary peak), which was simply a broad band. However, unlike AMCP, the primary peak for apatite had a gently sloping shoulder at the higher wave number side of the 900 to 1,200 cm\(^{-1}\) (primary peak), and the secondary peak was a composite of 2 peaks, which was a good match for the pattern of the apatite standard; this double peak was not present in the infrared spectroscopic pattern for AMCP. The XRD pattern of AMCP did not have distinct diffraction peaks, although some weak and broad peaks were recognized between 20 positions of 25° and 35°. These weak, broad peaks did not match any standard patterns in the International Centre for Diffraction Data database. The XRD pattern of apatite had some weak and broad diffraction peaks at 20 positions at which the most intense peaks of apatite standard were expected. However, the intensity and broadness of those peaks suggested that apatite from these urinary calculi was less crystalline, compared with the apatite standard. The infrared spectroscopic patterns for urinary calculi composed of struvite, calcium oxalate, or calcium carbonate all overlapped in the corresponding peaks of the standard as expected (data not shown); XRD of these samples confirmed the composition (data not shown).

Discussion

The results of this study showed the most common type of urinary calculus identified from purebred and crossbred potbellied pigs was AMCP on the basis of infrared spectroscopy and confirmation by XRD. This finding is in contrast to the reported mineral composition from previous studies in commercial pigs that included calcium carbonate, calcium oxalate, xanthine, uric acid, and urate. Furthermore, the study findings highlight the need for additional techniques such as infrared spectroscopy and possibly XRD to confirm AMCP in urinary calculi. To our knowledge, information regarding urinary calculi composition in potbellied pigs has not previously been reported. In the present study, a new infrared spectroscopic pattern for urinary calculi was found and defined as AMCP, which had previously been reported as apatite. In the University of California-Davis Stone Laboratory, previous analysis of AMCP from urinary calculi of cattle and goats by an energy-dispersive x-ray spectrometer revealed that the major elemental constituents were magnesium, phosphorus, and oxygen and that some calcium and carbon were also contained as minor constituents.

Amorphous magnesium calcium phosphate is irregular, flaky, or wavy and pale brown. Lack of birefringence suggests that AMCP has an amorphous state, which is supported by lack of x-ray diffraction peaks. On polarized light microscopy, apatite appears amorphous. Because of the very low birefringence and the infrared spectroscopic pattern of apatite, it is quite similar to that of AMCP because both are phosphates. Because of this, in earlier analyses AMCP was originally classified as apatite. Additionally, infrared spectroscopic patterns for AMCP have characteristics of both amorphous
calcium phosphate and struvite. Furthermore, when evaluated by polarized light microscopy, some AMCP aggregates are rectangular or octagonal in shape, which are characteristics of struvite crystals. These observations suggested that AMCP may be in a transition state from amorphous phosphate to struvite because AMCP does not diffract x-rays as crystals do. It is possible that AMCP is a precursor to struvite and could be named amorphous magnesium ammonium phosphate. It is essential to fully determine the chemical composition of AMCP; further investigation into the chemical composition of AMCP is warranted to help structure prevention strategies for pigs and other species that may be predisposed to formation of AMCP urinary calculi.

In addition to AMCP, the urinary calculi from this population of potbellied pigs also included calculi composed of struvite, calcium oxalate, and calcium carbonate. These minerals were confirmed by all 3 analytic methods. Calcium oxalate and calcium carbonate urinary calculi have previously been reported in commercial pigs; however, unlike in commercial pigs, urinary calculi composed of urate were not identified in potbellied pigs of the present study. One possible explanation for this difference is the age of the pigs. Although urate was reported in preweaned piglets, the median age for the potbellied pigs of the present study was 4 years old.

Relatively few samples from potbellied pigs were submitted over this 29-year period. In contrast, from 1985 through 2006, 25,499 canine urinary calculi were submitted for analysis to the same laboratory. This finding suggested that few urinary calculi from potbellied pigs are submitted for analysis or alternatively that urolithiasis is uncommon in potbellied pigs. Additionally, dogs are more often kept as pets than potbellied pigs.

Risk factors for development of urolithiasis in commercial pigs include reduced water intake, high or low urinary pH, imbalances in the diet, urinary stasis, and preexisting urinary tract infections. It is anticipated that these same risk factors may also play a role in development of urolithiasis in potbellied pigs. In the present study, castrated males were overrepresented, relative to females. This finding is consistent with that of other studies in dogs, cats, horses, cattle, sheep, goats, and pigs. The combination of a longer and smaller diameter of the urethra in castrated males versus females, and not necessarily any other specific predisposition for males to form urinary calculi, may have accounted for this sex predilection.

In the present study, most urinary calculi submitted for analysis had ≥ 2 minerals identified in each layer. Thus, identifying the mineral composition of a urinary calculus by grossly examining the outer layer only at the time of removal may lead to insufficient recommendations in preventing urolithiasis in potbellied pigs, and it is recommended that calculi be submitted for a complete mineral analysis whenever possible. As a result of the small sample size, the relationship between age and location of the urinary calculus in the urinary tract at the time of removal or between urinary calculus type and location of the urinary calculus at the time of removal was not investigated in the present study. It is also important to interpret the findings of urinary calculus location with caution because urinary calculi recovered from the urinary bladder were overrepresented. Urinary calculus recovered from the urinary bladder alone or urethra alone or from both sites were submitted for analysis. Thus, the frequency of urinary calculi from the urinary bladder and urethra are confounded in that urinary calculi found in the urethra are most likely to be from the urinary bladder but less likely vice versa.

General recommendations to prevent urolithiasis in pigs include adequate water intake, adequate balanced mineral diet, and reducing lower urinary tract infections. Acidification of urine with oral administration of ammonium chloride has been recommended for prevention of struvite and calcium phosphate urinary calculi in goats, sheep, horses, cats, and dogs. If AMCP is a precursor to struvite, acidification of the urine may be beneficial in preventing these urinary calculi as well. In our teaching hospital, oral administration of methionine for urine acidification in pigs is recommended.

References