Iodinated non-ionic contrast medium does not inhibit *Escherichia coli* growth in ex vivo inoculated canine urine

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OBJECTIVE
To investigate the effect of iohexol on standardized quantitative urine culture results in dogs. The authors hypothesized that the presence of iohexol in inoculated urine samples would result in lower bacterial concentrations (CFU/mL) and, therefore, decrease culture sensitivity.

SAMPLE
Urine samples were aseptically collected during cystoscopy from a single client-owned dog untreated with antimicrobials.

PROCEDURES
An experimental controlled study. The urine sample was divided into 38 aliquots (0.5 mL each) that were used as negative controls or inoculated with an equal amount of *Escherichia coli* (10^5 CFU/mL). Different volumes (0.1 and 0.5 mL) of contrast or saline were added to the aliquots and quantitative culture results were compared. Two different incubation times between the preparation of aliquots and culture were evaluated (15 minutes and 24 hours).

RESULTS
All aliquots from samples inoculated with *E. coli* (positive controls and iohexol-group) had the same reported quantitative result (10^4 CFU/mL). No growth was reported for the negative controls. Iohexol did not show any anti-*E. coli* properties in canine urine cultures for dilutions up to 1:2 contrast:urine and concentrations up to 120 mgI/mL. No difference was reported when iohexol was incubated with inoculated urine for 15 minutes or 24 hours.

CLINICAL RELEVANCE
Based on the experimental in vitro conditions described, administration of iohexol before the collection of urine during urologic procedures does not negatively impact the isolation and growth of *E. coli*.

The effect of iodinated contrast media on bacterial growth has been investigated in human medicine. However, evidence from scientific studies appears to be contradictory and evidence in veterinary medicine is lacking. Several studies in the human literature report that iodinated contrast agents exert an inhibitory effect on bacterial growth, whereas other studies do not support those conclusions. Iodinated contrast media can be placed into 2 categories: ionic (eg, sodium or meglumine diatrizoate, sodium or meglumine iothalamate, sodium acetrizoate, and sodium metrizoate) and non-ionic (eg, iohexol, iopamidol, and metrizamide). Among the studies using ionic contrast media, one study investigated their effect on urinary tract pathogens in humans. Their results indicated that sodium acetrizoate and sodium diatrizoate were bactericidal for *Escherichia coli*. A study by Narins et al found that sodium diatrizoate was bactericidal for *E. coli* in nutrient broth; however, this inhibitory effect was not observed in naturally infected urine. A study performed a couple of years later by Leland et al concluded that iodinated ionic contrast agents did not significantly inhibit bacterial growth under in vitro conditions corresponding to standard laboratory processing. Two studies compared the antibacterial effects of ionic and non-ionic contrast media on urinary pathogens. Johansen et al concluded that contrast media had no bactericidal effect and only had a negligible bacteriostatic effect. Dawson et al demonstrated a bactericidal and bacteriostatic effect of contrast media in vitro;
however, they found that this inhibitory effect was neutralized by urine.6

Currently, low osmolarity, iodinated, non-ionic contrast media, such as iohexol, are mostly used in veterinary interventional radiology.7 Textbooks in veterinary medicine recommend that urine samples should be collected for culture before injecting iodinated contrast medium during nephroptogram and suggest that if contrast is present in the sample, bacterial growth can be affected and culture results can be falsely negative.2,3 To the authors’ knowledge, the effect of iohexol on bacterial growth in canine or feline urine samples has not been investigated.

The primary goal of this study was to evaluate the effect of an iodinated, non-ionic contrast medium (iohexol) on quantitative urine culture results in dogs by comparing bacterial growth in urine samples inoculated with Escherichia coli in the presence or absence of contrast dilutions for different periods. The authors hypothesized that the presence of iohexol in inoculated urine samples would result in lower bacterial concentrations (CFU/mL) and, therefore, decrease culture sensitivity.

Materials and Methods

Urine collection

This experimental controlled study was conducted at the Purdue University Veterinary Hospital and the Indiana Animal Disease Diagnostic Laboratory. Residual urine was collected during cystoscopy of a 6-year-old, female spayed, client-owned mixed breed dog who presented to the hospital for dysuria. A complete workup including hematology, serum biochemistry, urinalysis, aerobic urine culture (collected via cystocentesis), abdominal imaging (radiographs and ultrasound), and cystoscopic examination was completed and revealed no significant abnormalities. Bladder biopsies were performed and submitted for aerobic bacterial culture and Mycoplasma sp. PCR and both results were negative. A total of 20 mL of urine was collected during the cystoscopy and stored at 4°C in tubes with no additive for 24 hours before the study. The authors were careful not to infuse any saline as they were passing the scope into the bladder to avoid dilution of the sample.

Aliquot preparation

The original urine sample was divided into 0.5 mL aliquots that were then prepared for quantitative bacterial culture. The composition of each aliquot is summarized (Table 1). The addition of a letter to the aliquot group number indicates either the different volume of dilution or inoculation-to-culture time used within the same aliquot group. The aliquot group 1 was used as a negative control. The aliquot groups 2a and 2b were used to confirm that the addition of volumes of saline (equivalent to the volumes of contrast added to the urine aliquot) did not cause bacterial contamination. The aliquot groups 3a and 3b were used to confirm that the addition of different volumes of iohexol did not cause bacterial contamination. The aliquot group 4 was used as a positive control. The aliquot group 5 was made by adding different volumes of 0.9% sodium chloride solution (aliquot 5a: 0.1 mL; aliquot 5b and aliquot 5c: 0.5 mL) to inoculated urine to evaluate the effect of dilution on quantitative culture results. Finally, the aliquot group 6 was made by adding equivalent volumes of iohexol (Omnipaque [240 mgI/mL]; GE Healthcare) to inoculated urine (aliquot 6a: 0.1 mL; aliquot 6b and aliquot 6c: 0.5 mL). Two different times between inoculation and culture were evaluated. The aliquot groups 5c (urine, Escherichia coli, and saline) and 6c (urine, Escherichia coli, and iohexol) were stored in the refrigerator at 4°C and cultured 24 hours later, while all the other aliquots were cultured within 15 minutes of preparation of the final dilution. The prolonged storage time was chosen to mimic clinical conditions where a sample may be stored or shipped overnight before inoculating culture media.

E. coli culture and volume of inoculation

Thioglycollate broth (Remel) was inoculated with 1 colony of Escherichia coli (ATCC 25922) and incubated

Table 1—Characteristics of aliquots and concentration of Escherichia coli (CFU/mL) obtained for each aliquot studied.

<table>
<thead>
<tr>
<th>Aliquot group</th>
<th>Composition</th>
<th>iohexol concentration (mgI/mL)</th>
<th>Inoculation-to-culture time (min)</th>
<th>Number of aliquots</th>
<th>Quantitative urine culture (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Urine (0.5 mL)</td>
<td>0</td>
<td>15 min</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1b</td>
<td>Urine (0.5 mL)</td>
<td>0</td>
<td>24 h</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2a</td>
<td>Urine (0.5 mL) + saline (0.1 mL)</td>
<td>0</td>
<td>15 min</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2b</td>
<td>Urine (0.5 mL) + saline (0.5 mL)</td>
<td>0</td>
<td>15 min</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3a</td>
<td>Urine (0.5 mL) + iohexol (0.1 mL)</td>
<td>40</td>
<td>15 min</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3b</td>
<td>Urine (0.5 mL) + iohexol (0.5 mL)</td>
<td>120</td>
<td>15 min</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Urine (0.5 mL) + E. coli (10⁵ CFU/mL)</td>
<td>0</td>
<td>15 min</td>
<td>2</td>
<td>10⁴</td>
</tr>
<tr>
<td>5a</td>
<td>Urine (0.5 mL) + E. coli (10⁵ CFU/mL) + saline (0.1 mL)</td>
<td>0</td>
<td>15 min</td>
<td>5</td>
<td>10⁴</td>
</tr>
<tr>
<td>5b</td>
<td>Urine (0.5 mL) + E. coli (10⁵ CFU/mL) + saline (0.5 mL)</td>
<td>0</td>
<td>15 min</td>
<td>5</td>
<td>10⁴</td>
</tr>
<tr>
<td>5c</td>
<td>Urine (0.5 mL) + E. coli (10⁵ CFU/mL) + saline (0.5 mL)</td>
<td>0</td>
<td>24 h</td>
<td>5</td>
<td>10⁴</td>
</tr>
<tr>
<td>6a</td>
<td>Urine (0.5 mL) + E. coli (10⁵ CFU/mL) + iohexol (0.1 mL)</td>
<td>40</td>
<td>15 min</td>
<td>5</td>
<td>10⁴</td>
</tr>
<tr>
<td>6b</td>
<td>Urine (0.5 mL) + E. coli (10⁵ CFU/mL) + iohexol (0.5 mL)</td>
<td>120</td>
<td>15 min</td>
<td>5</td>
<td>10⁴</td>
</tr>
<tr>
<td>6c</td>
<td>Urine (0.5 mL) + E. coli (10⁵ CFU/mL) + iohexol (0.5 mL)</td>
<td>120</td>
<td>24 h</td>
<td>5</td>
<td>10⁴</td>
</tr>
</tbody>
</table>

CFU = colony forming units. The incubation temperature for each mixture was 36°C ± 2°C.
at 37 °C for 24 hours. A broth concentration of 5 X 10⁸ CFU/mL was obtained. This broth was then used to inoculate aliquots of urine and achieve a concentration of 10⁴ CFU/mL of *E. coli* in each aliquot (Table 1).

**Quantitative urine culture**

Quantitative urine cultures were performed for each aliquot group according to the Indiana Animal Disease Diagnostic Laboratory standard operating procedure. Identification and susceptibility testing was performed by properly trained staff. For the aliquot groups 1, 2, 3, and 4 (negative and positive controls), bacterial cultures were repeated twice, whereas for the aliquot groups 5 and 6, bacterial cultures were repeated 5 times (Table 1). For each culture, a 1 µL loop was used to inoculate 1 blood agar plate (BAP) and 1 MacConkey agar plate (MAC). The process was repeated using a 10 µL loop. BAP was incubated at 36 °C in 5% CO₂, and MAC was incubated at 36 °C in room air. BAP was examined at 24 hours and 48 hours and MAC was examined at 24 hours. The concentration reported by the laboratory (CFU/mL) was based on the number of colonies counted per plate as described (Table 2).

### Table 2—Reported concentration of colony forming units (CFU/mL) based on the number of colonies counted as per the Indiana Animal Disease Diagnostic Laboratory standard operating procedure.

<table>
<thead>
<tr>
<th>CFU/mL</th>
<th>1 µL plate</th>
<th>10 µL plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10³</td>
<td>1–9 colonies</td>
<td>10–99 colonies</td>
</tr>
<tr>
<td>10³</td>
<td>10–99 colonies</td>
<td>100–999 colonies</td>
</tr>
<tr>
<td>10⁴</td>
<td>≥ 100 colonies</td>
<td>≥ 1,000 colonies</td>
</tr>
</tbody>
</table>

**Results**

No growth was reported for aliquots 1, 2, and 3 (negative controls), and all aliquots from samples inoculated with *E. coli* had the same reported quantitative result (Table 1). Between 10 and 99 colonies were counted on all 1 µL BAP and MAC plates for the aliquots 4 (positive controls), 5 (saline addition), and 6 (iohexol addition), equivalent to 10⁴ CFU/mL. There was no difference between the multiple plates in each aliquot group. The aliquots with different duration of exposure to iohexol (15 minutes or 24 hours) also had the same reported quantitative result. No quantitative difference was reported when BAP was examined at 24 hours and 48 hours.

**Discussion**

The present study reports the effect of iohexol, an iodinated non-ionic contrast medium, on bacterial growth in canine urine inoculated with *E. coli*. In the authors’ hospital, iohexol is most commonly used as a contrast medium during interventional urology procedures and other imaging studies (eg, CT scans). Under the experimental conditions described, iohexol did not impact the results of quantitative cultures in canine urine inoculated with *E. coli*. The initial proportions of urine and contrast in the subgroups 1 (Table 1) were chosen based on an average canine bladder volume of 10 mL/kg, as previous studies suggested 3.5 mL/kg being a normal bladder volume and up to 20 mL/kg as maximum bladder volume.5,12 While a safe contrast dosage has not been determined for urologic procedures, 2 mL/kg is a commonly used dosage during standard intra-vascular interventional procedures.7 By dividing this dose (2 mL/kg) by the average bladder volume of a dog (10 mL/kg), the authors elected to use 0.1 mL of contrast for 0.5 mL of urine for the subgroups a. In vivo, the bladder volume of a dog varies based on urine production, and the proportion of contrast in the bladder of a dog undergoing CT angiography or endourologic procedures is generally undetermined. Hence, the contrast-to-urine ratio in our study may not correlate with the dilution of contrast naturally occurring with urine in the bladder.

Two different contrast concentrations were evaluated with aliquots 6a containing only 40 mgI/mL, and aliquots 6b and 6c containing 120 mgI/mL (equivalent to a 1:2 dilution). Culture results revealed that the concentration of iohexol did not impact the results of quantitative cultures. Additionally, culture results from sub-group 6c suggest that prolonged contact of urine with iohexol (over a 24-hour period) also does not seem to influence the reliability of urine culture results. The study design included 2 different incubation periods (15 minutes and 24 hours) to reflect the logistical aspect of urine culture submission in practice, as most laboratory centers have a cut-off time, after which, urine samples are processed the following day. The effect of dilution alone was also evaluated, by adding saline to an inoculated sample. Culture results from groups 4 and 5 suggest that dilution with saline up to a ratio of 1:2 does not influence results under the experimental conditions described. Overall, these results are consistent with previous studies performed in humans.5,12 This study has several limitations. First, a colony count was performed for each aliquot, but the results were reported as concentrations (CFU/mL; Table 2). When using a 1 µL loop, any colony count between 10 and 99 per plate was attributed a value of 10⁴ CFU/mL. Therefore, it is possible that our study may have missed some inhibition of bacterial growth. However, colony counts less than a log difference are not considered clinically significant. When a urine sample is submitted for culture to the Indiana Animal Disease Diagnostic Laboratory at Purdue University, culture results are reported as concentrations and not colony count per plate. Therefore, subtle inhibition of bacterial growth would not change the reported results and is unlikely to be clinically significant. Second, iodine is a broad-spectrum anti-septic agent and it has been shown that slight deiodination may occur in vivo or when contrast media are exposed to sunlight.13,14 Deiodination consists of partial reactions.
for the release of iodine atoms. In our study, an increased iodine concentration after deiodination could have increased the potential bacteriostatic or bactericidal effect of the contrast solution and hampered bacterial growth, but no quantitative difference was reported between aliquots mixed with iohexol and the positive controls. Moreover, the contrast was stored and used according to the manufacturer’s recommendations. Measurements of iodine and iodide in the aliquots 6 were deemed outside the scope of the current investigation. Third, a urinalysis was performed on the original urine sample collected via cystocentesis, but a urinalysis of the urine sample collected via cystoscopy the following day was not repeated. Although some urine dilution could have happened during cystoscopy, both negative and positive control results were adequate and did not reveal change due to any variable in the experiment, which supports that the experiment was performed properly. The osmolality of each aliquot was not measured; however, a study reported that the osmolality of a culture medium did not affect the growth of E. coli.4 Fourth, this study evaluated 2 concentrations of iohexol (40 mg/mL and 120 mg/mL). Higher concentrations of iohexol could have potentially resulted in different effects. However, Johansen et al exposed urinary pathogens in vitro to iodinated and non-ionic contrast agents (sodium metrizoate and meglumine, respectively) in concentrations of 100 and 260 mg/mL and reported that both contrast media had a slight or negligible bacteriostatic effect on the test bacteria and no bactericidal effect was detected. They concluded that radiography of the urinary tract with these 2 media in the concentrations measured; however, a study reported that the osmolality of a culture medium did not affect the growth of E. coli.4 However, this study only evaluated the effect of iohexol dilution on 1 strain of 1 type of bacteria (E. coli). Testing different bacterial strains or bacterial strains of E. coli could have led to different results. In the human literature, several authors reported that the bactericidal effect of contrast agents varies between bacterial strains.2,6 The ATCC 25922 E. coli strain was selected for this study because it was in use in the ADDL as a quality control organism for antimicrobial susceptibility testing of veterinary isolates per the Clinical and Laboratory Standards Institute VET01S manual. While originally isolated from a human clinical sample, the authors anticipated it to approximate a veterinary isolate in the laboratory. However, this study design could be repeated with a canine-derived strain of E. coli or other uropathogens. Finally, this study only evaluated ex vivo inoculated urine from 1 dog. There could be differences in urine composition that might influence how iohexol interacts with bacteria, including pH values, medications, proteinuria, or other substances that might interact with iodine. Previous studies suggested different bactericidal and bacteriostatic activities of iodinated contrast media in vivo compared with in vitro.3,5,6 Dawson et al investigated the effects of some constituents of urine (urea, bile acid, hydrocortisone, and adrenalin) on the bactericidal action of an iodinated ionic contrast agent (iothalamate meglumine) against E. coli but none of them seemed to affect its activity.6 To conclude, under the experimental conditions described in the manuscript, iohexol did not show any anti-E. coli activity for concentrations up to 120 mg/mL and dilutions up to 1:2 contrast:urine. Moreover, 24 hours of contact with iohexol with inoculated urine did not influence the reported urine culture results. Additionally, dilutions up to 1:2 saline:urine did not affect the reported urine culture results either.

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