

Effects of cranberry extract on prevention of urinary tract infection in dogs and on adherence of *Escherichia coli* to Madin-Darby canine kidney cells

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OBJECTIVE

To determine effects of cranberry extract on development of urinary tract infection (UTI) in dogs and on adherence of *Escherichia coli* to Madin-Darby canine kidney (MDCK) cells.

ANIMALS

12 client-owned dogs (in vivo experiment) and 6 client-owned dogs (in vitro experiment).

PROCEDURES

12 dogs with a history of recurrent UTI received an antimicrobial (n = 6) or cranberry extract (6) orally for 6 months. Dogs were monitored for a UTI. For the in vitro experiment, cranberry extract was orally administered to 6 dogs for 60 days. Voided urine samples were collected from each dog before and 30 and 60 days after onset of extract administration. Urine was evaluated by use of a bacteriostasis assay. An antiadhesion assay and microscopic examination were used to determine inhibition of bacterial adherence to MDCK cells.

RESULTS

None of the 12 dogs developed a UTI. The bacteriostasis assay revealed no zone of inhibition for any urine samples. Bacterial adherence was significantly reduced after culture with urine samples obtained at 30 and 60 days, compared with results for urine samples obtained before extract administration. Microscopic examination revealed that bacterial adherence to MDCK cells was significantly reduced after culture with urine samples obtained at 30 and 60 days, compared with results after culture with urine samples obtained before extract administration.

CONCLUSIONS AND CLINICAL RELEVANCE

Oral administration of cranberry extract prevented development of a UTI and prevented *E coli* adherence to MDCK cells, which may indicate it has benefit for preventing UTIs in dogs. (*Am J Vet Res* 2016;77:421–427)

Urinary tract infections are associated with a temporary or permanent breach in host defense mechanisms that allows virulent microbes to adhere, multiply, and persist within the urinary tract. Infections can be confined to a single site within the urogenital tract, such as the renal pelvis (pyelonephritis), ureter (ureteritis), bladder (cystitis), urethra (urethritis), prostate gland (prostatitis), or vagina (vaginitis), or can be found at multiple sites.¹ Although fungi and viruses also infect the urinary tract, UTIs are most commonly caused by bacteria such as *Escherichia coli*, which is the most common uropathogen.^{2–4}

Cranberries consist primarily of water (88%), but they also contain organic acids (including salicylate), fructose, high amounts of vitamin C (200 mg/kg of fresh berries), flavonoids, proanthocyanidins, cate-

chins, and triterpenoids.^{4,5} The *E coli* strains that cause UTIs have proteinaceous macromolecules (fimbriae) that facilitate adhesion of bacteria to uroepithelial cells in the urinary tract. In vitro and in vivo studies^{5,6} indicate that cranberry products prevent bacterial adhesion to cells in the wall of the urinary tract.

Studies^{5,7} indicate that the consumption of cranberry extract can prevent UTIs in women. However, there is a paucity of studies on the benefits of cranberries for prevention of UTIs in dogs. Therefore, the objectives of the study reported here were to investigate the effect of cranberry extract on the development of UTIs in dogs with a history of recurrent UTIs and to evaluate effects of urine obtained from dogs provided cranberry extract on adherence of *E coli* to MDCK cells.

Materials and Methods

Animals

The in vivo experiment involved 12 client-owned female dogs, each of which had at least 3 UTIs during

ABBREVIATIONS

DMEM	Dulbecco modified Eagle medium
MDCK	Madin-Darby canine kidney
UTI	Urinary tract infection

the preceding year. All dogs were confirmed to have recovered from the most recent UTI, as determined on the basis of results of urinalysis and bacterial culture of a urine sample.

The *in vitro* experiment involved 6 client-owned dogs (4 mixed-breed dogs, 1 Pug, and 1 Shih Tzu). There were 3 neutered males and 3 spayed females. Age of the dogs ranged from 7 to 11 years (mean, 8.9 years), and body weight ranged from 6.5 to 20.5 kg (mean, 13.6 kg). All 6 dogs were considered healthy at the time of enrollment, as determined on the basis of the medical history and results of a complete physical examination (no signs of urinary tract disease).

Owners provided consent for inclusion of the dogs in the study. All dogs received care in accordance with institutional animal care and use committee guidelines.

Experimental design

In vivo experiment—Dogs were allocated into 2 groups (6 dogs/group). One group comprised 4 Schnauzers and 2 Toy Poodles (3 spayed and 3 sexually intact; age of the dogs ranged from 7 to 14 years [mean, 9.8 years], and body weight ranged from 2.8 to 7.4 kg [mean, 5.6 kg]). These dogs received cephalexin^a (20 mg/kg, PO, q 12 h for 14 days). The second group comprised 4 Schnauzers and 2 Chihuahuas (4 spayed and 2 sexually intact; age of the dogs ranged from 5 to 12 years [mean, 8.0 years], and body weight ranged from 2.5 to 7.3 kg [mean, 5.3 kg]). Dogs in the second group received powdered cranberry extract^b daily for 6 months. The powder was mixed with food and administered to each dog at the morning meal. The amount of cranberry extract provided to each dog was the dose specified on the product (1 g for dogs < 25 kg and 2 g for dogs ≥ 25 kg). The first day of administration of cephalexin or cranberry extract was designated as day 1.

Dogs were monitored throughout the experiment. Blood samples and voided urine samples were collected from each dog immediately before onset of cephalexin or cranberry extract administration and then once per month for 6 months. Once each month, a complete physical examination, hematologic examination, biochemical analysis, urinalysis, and bacterial culture of a urine sample were performed.

In vitro experiment—Dogs received powdered cranberry extract^b (1 g for dogs < 25 kg and 2 g for dogs ≥ 25 kg) daily for 60 days (1 day before administration of cranberry extract was designated as day 0). The powder was mixed with food and administered to each dog at the morning meal. Voided urine samples were collected from each dog immediately before onset of cranberry extract administration and on days 30 and 60.

Preparation of urine samples

Urine samples were collected in the morning and centrifuged at 1,000 X *g* for 5 minutes to precipitate

particulate matter. Supernatant was removed with a sterile Pasteur pipette and vacuum-filtered by use of a commercial filtration unit with a 0.22- μ m polyethersulfone filter^c; filtered urine was collected in a sterile 50-mL conical tube and frozen at -20°C for use in a bacteriostasis assay.

Propagation of uropathogenic *E coli* strains and preparation of bacterial suspensions

Three uropathogenic *E coli* strains (C1-50, C2-48, and C3-48) were isolated from dogs with UTI examined at the Veterinary Medical Teaching Hospital of the National Chung Hsing University. The *E coli* strains were grown on blood agar plates at 35°C for 24 to 48 hours. After distinct bacterial colonies appeared, the plates were sealed and stored at 4°C until used for the bacteriostasis assay.

An *E coli* colony was selected; it was then streaked onto trypticase soy agar^d and incubated overnight at 35°C. The next morning, *E coli* were suspended in 3 or 5 mL of saline (0.9% NaCl) solution. A standard bacterial concentration of 10⁶ CFUs/mL, as determined by use of a 0.5-McFarland standard,⁸ was used for the bacteriostasis assay.

Bacteriostasis assay

A swab specimen of the bacterial suspension was smeared onto plates containing Mueller-Hinton agar.^e Seven holes were punched in each agar plate; 1 hole was filled with 100 μ L of sterile saline solution (negative control sample), and the remaining 6 holes were each filled with 100 μ L of the urine sample of 1 dog at 1 time point. A disk containing enrofloxacin^f was used as the positive control sample. A positive result was considered to be an inhibition zone with a diameter > 21 mm. Plates were incubated at 35°C for 24 hours, and the inhibition zone around each hole was then assessed.

Preparation of MDCK cells

The MDCK cells were obtained from the Graduate Institute of Veterinary Pathobiology at National Chung Hsing University. They were maintained in DMEM^g that contained 4.5 g of glucose/L, sodium pyruvate, and 4mM stable glutamine and 10% (vol/vol) heat-inactivated fetal bovine serum supplemented with 1mM sodium pyruvate and 1% (vol/vol) antimicrobial (penicillin, streptomycin, and amphotericin B) solution. Stock cultures of cells were propagated in 75-cm² plastic flasks at 37°C in a humidified 95% O₂-5% CO₂ atmosphere and passaged as needed.

Antiadhesion assay

The efficacy of cranberry extract for inhibiting bacterial adherence to MDCK cells was evaluated by use of an *in vitro* assay with modifications described elsewhere.⁹ Antiadhesion assays were performed as follows.

The MDCK cells that had grown to confluence at 37°C were placed in 96-well plastic plates (10⁴ cells/well) for the antiadhesion assay. Culture media were discarded, and each well was washed with PBS solution (100 μL). The wash solution was discarded, and plates were tapped dry on absorbent paper. Immediately before the assay, MDCK cells were fixed with 5% methanol. An aliquot (100 μL) of methanol was added to each well, and plates were allowed to sit undisturbed for 2 minutes. The methanol was then discarded, and plates were tapped dry on absorbent paper. Plates were then further dried in a laminar flow hood for 10 minutes.

A test sample of urine plus bacteria was created by mixing an aliquot of the bacterial suspension (a standard bacterial concentration of 10⁶ CFUs/mL, as determined by use of a 0.5-McFarland standard⁸) with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration. The ratio was 1:10 (1 part bacterial suspension to 9 parts urine sample). Each well of a 96-well plastic plate was prepared by adding 50 μL of the test sample (urine plus bacteria) and 150 μL of DMEM (final volume, 200 μL/well) to the methanol-fixed MDCK cells. Plates were then incubated at 25°C for 30 minutes.

After incubation was complete, the plates were incubated for an additional 60 minutes at 35°C to permit

bacterial attachment. After this 60-minute incubation was complete, nonadhered bacteria and media were removed by aspiration, and the wells were rinsed 3 times with PBS solution (200 μL/rinse). Then, 200 μL of DMEM plus 5% heat-inactivated fetal bovine serum was added to each well. Plates were incubated at 35°C for 18 hours to allow growth of attached bacteria. After the 18-hour incubation was complete, absorbance for each well was determined at 650 nm by use of a microplate reader^h and commercial software.ⁱ

Microscopic examination

The MDCK cells that had grown to confluence at 37°C were placed in 24-well plastic plates (5 × 10⁴ cells/well) for the antiadhesion assay. Culture media were discarded, and each well was washed with PBS solution (100 μL). The wash solution was discarded, and plates were tapped dry on absorbent paper. Immediately before the assay, MDCK cells were fixed with 5% methanol. An aliquot (200 μL) of methanol was added to each well, and plates were allowed to sit undisturbed for 2 minutes. The methanol was then discarded, and plates were tapped dry on absorbent paper. Plates were then further dried in a laminar flow hood for 10 minutes.

A test sample of urine plus bacteria was created by mixing an aliquot of the bacterial suspension (a standard bacterial concentration of 10⁶ CFUs/mL, as determined by use of a 0.5-McFarland standard⁸) with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration. The ratio was 1:10 (1 part bacterial suspension to 9 parts urine sample). Each well of a 24-well plastic plate was prepared by adding 200 μL of the test sample (urine plus bacteria) and 300 μL of DMEM (final volume, 200 μL/well) to the methanol-fixed MDCK cells. Plates were then incubated at 25°C for 30 minutes.

After the initial incubation was complete, the plates were incubated for an additional 3 hours. Slides were stained with crystal violet and examined microscopically (1,000X magnification).

Statistical analysis

All data were expressed as mean ± SEM. Differences between groups were tested by use of the Student *t* test. Values of *P* < 0.05 were considered significant. Linear regression analysis was used to evaluate results for the antiadhesion assay and microscopic examination.

Results

In vivo experiment

None of the 12 dogs developed a UTI during the experimental period

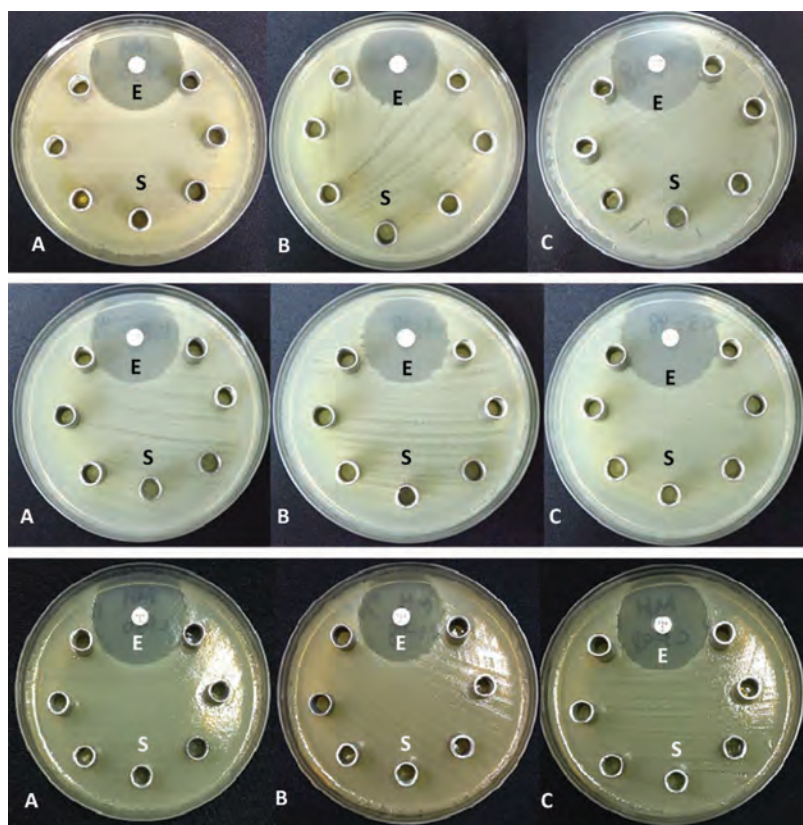


Figure 1—Photographs of a bacteriostasis assay against *Escherichia coli* strains C1-50(A), C2-48 (B), and C3-48 (C) cultured with urine samples obtained from a representative dog before (top row) and 30 (middle row) and 60 (bottom row) days after onset of cranberry extract administration. Notice that the inhibition zone for the positive control sample (enrofloxacin [E]) is > 30 mm, which indicates an inhibitory effect. Sterile saline (0.9% NaCl) solution (S) is the negative control sample.

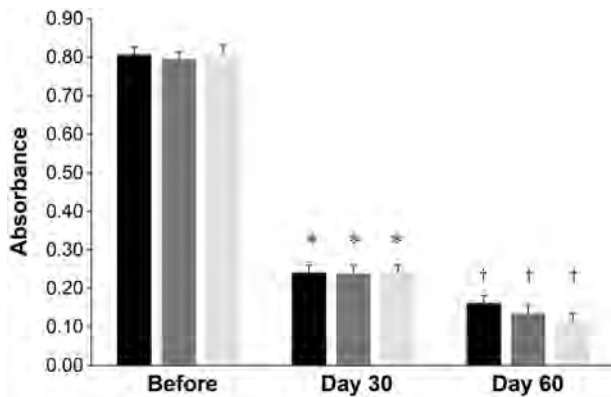


Figure 2—Mean ± SEM absorbance for *E coli* strain C1-50 (black bars), C2-48 (dark gray bars), and C3-48 (light gray bars) after culture in MDCK cells with urine samples obtained from 6 dogs before and 30 and 60 days after onset of cranberry extract administration. *Within a strain, value differs significantly ($P < 0.05$) from the value after culture with the urine samples obtained before the onset of cranberry extract administration. †Within a strain, value differs significantly ($P < 0.05$) from the values after culture with urine obtained before and 30 days after onset of cranberry extract administration.

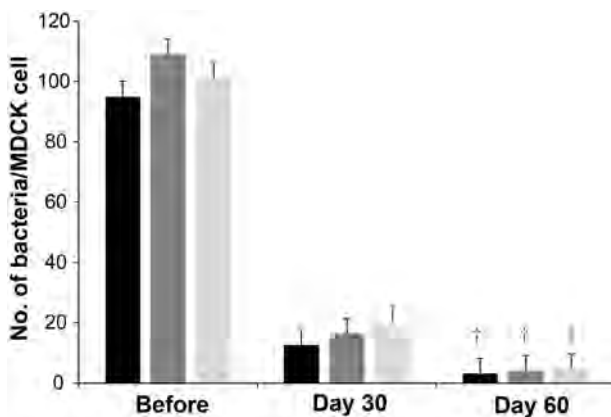


Figure 3—Mean ± SEM number of *E coli* (strain C1-50, C2-48, and C3-48) adhered to MDCK cells after culture with urine samples obtained from 6 dogs before and 30 and 60 days after onset of cranberry extract administration. See Figure 2 for key.

as determined on the basis of a lack of clinical signs and laboratory results (especially results for bacterial culture of a urine sample) that yielded no evidence of infection. On the basis of the medical history of these dogs, 12 cases of UTI (95% confidence interval, 10 to 14 cases of UTI) would have been expected during the 6-month experimental period. No adverse effects attributable to treatment were reported.

In vitro experiment

Results of the bacteriostasis assay were the same for urine samples obtained before and 30 and 60 days after the onset of cranberry extract administration to the 6 dogs. Enrofloxacin (positive control sample)

yielded the only inhibition zone (diameter > 30 mm). No inhibition zone was observed around the sterile saline solution (negative control sample) or the urine samples of the 6 dogs (**Figure 1**).

Bacterial adhesion was reduced for urine samples obtained at 30 and 60 days from each of the 6 dogs, compared with results for the urine sample obtained before administration of cranberry extract. Mean ± SEM absorbance for C1-50 *E coli* cultured in plates containing MDCK cells with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration was 0.81 ± 0.03 , 0.24 ± 0.01 , and 0.16 ± 0.02 , respectively (**Figure 2**). Mean ± SEM absorbance for C2-48 *E coli* cultured in plates containing MDCK cells with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration was 0.80 ± 0.03 , 0.24 ± 0.01 , and 0.14 ± 0.02 , respectively. Mean ± SEM absorbance for C3-48 *E coli* cultured in plates containing MDCK cells with urine samples obtained before and 30 and 60 days after onset of cranberry extract administration was 0.81 ± 0.02 , 0.24 ± 0.02 , and 0.12 ± 0.01 , respectively. Mean absorbance for the 3 *E coli* strains cultured with MDCK cells and urine samples obtained at 30 and 60 days was significantly lower than the absorbance for culture with the urine sample obtained before onset of cranberry extract administration. Moreover, mean absorbance of the 3 *E coli* strains cultured with MDCK cells and urine obtained at 60 days was also lower than that for urine samples obtained before and at 30 days after onset of administration of cranberry extract.

Adherence of the 3 *E coli* strains was decreased from a mean of 101.84 adherent bacteria/MDCK cell after incubation with urine samples obtained before cranberry extract administration to 16.44 and 4.00 adherent bacteria/MDCK cell after incubation with urine samples obtained at 30 and 60 days, respectively. Mean ± SEM number of C1-50 *E coli* adhering to MDCK cells was 95.17 ± 10.65 , 12.67 ± 3.5 , and 3.17 ± 2.04 for the urine samples obtained before and 30 and 60 days after onset of cranberry extract administration, respectively (**Figure 3**). Mean ± SEM number of C2-48 *E coli* adhering to MDCK cells was 109.17 ± 10.61 , 16.33 ± 3.5 , and 4.17 ± 2.64 for the urine samples obtained before and 30 and 60 days after onset of cranberry extract administration, respectively. Mean ± SEM number of C3-48 *E coli* adhering to MDCK cells was 101.17 ± 9.52 , 20.33 ± 3.56 , and 4.67 ± 1.86 for the urine samples obtained before and 30 and 60 days after onset of cranberry extract administration, respectively. Compared with the mean adherence for the urine sample obtained before onset of cranberry extract administration, the mean *E coli* adherence to the MDCK cells for the urine samples obtained at 30 and 60 days was significantly lower. Moreover, the mean *E coli* adherence to the MDCK cells was significantly lower for the urine sample obtained at 60 days than for the urine sample obtained at 30 days. Adherence of

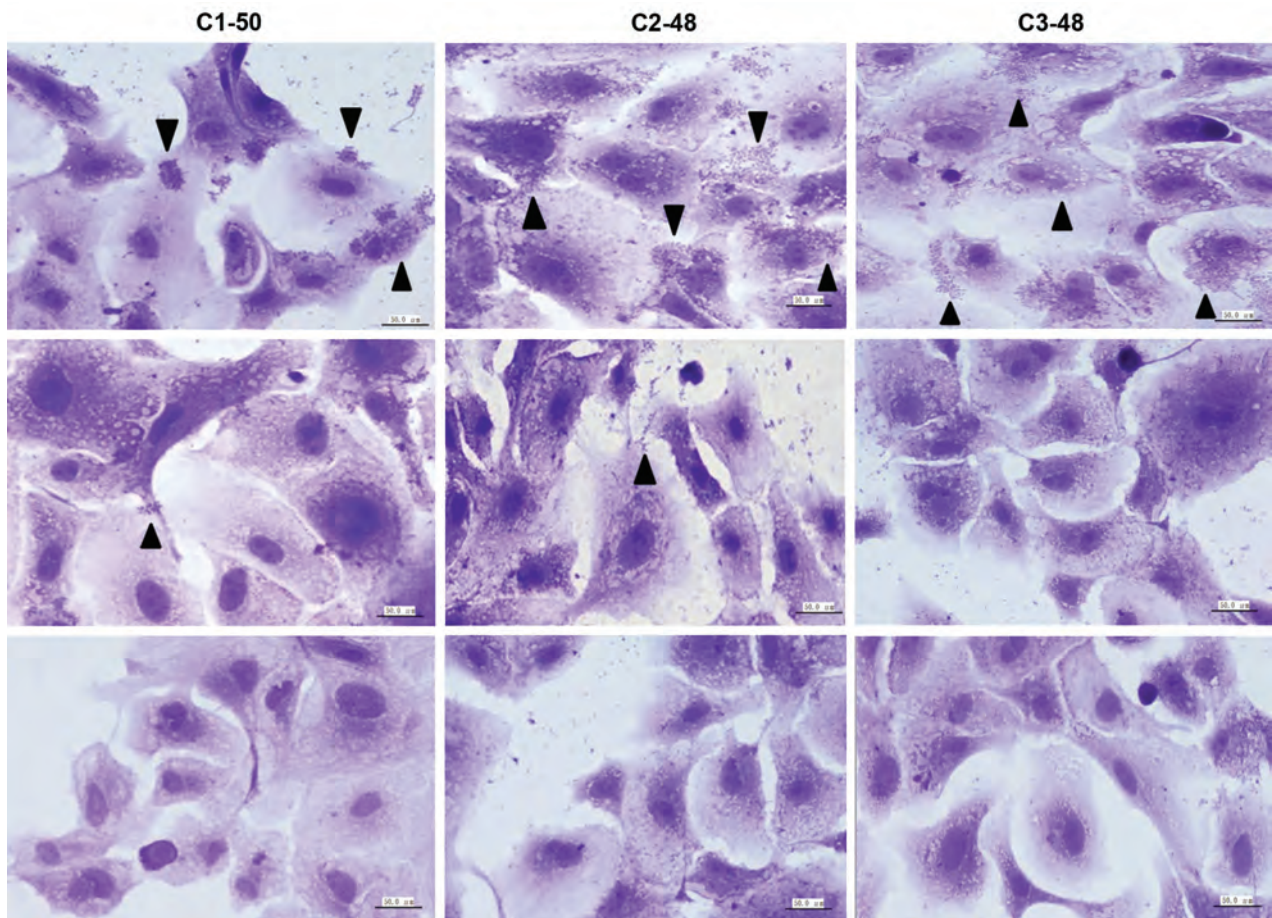


Figure 4—Photomicrographs of cultured MDCK cells indicating adherence of *E coli* strain C1-50, C2-48, and C3-48 to the cells after culture with urine samples obtained from a representative dog before (top row) and 30 (middle row) and 60 (bottom row) days after onset of cranberry extract administration. Notice the bacteria (arrowheads) that are adhered to the cells. Crystal violet stain; bar = 50 μ m.

E coli to MDCK cells was evident during microscopic examination (**Figure 4**).

Discussion

In the *in vivo* experiment reported here, an antimicrobial and powdered cranberry extract were administered to prevent UTIs in dogs. None of the dogs developed UTIs, as determined on the basis of clinical signs and laboratory results, which corresponded with results of another study.⁵ Some studies^{6,10,11} of humans indicate that the use of cranberries to prevent UTIs is better than the prophylactic use of low-dose antimicrobials because long-term use of antimicrobials increases the risk of antimicrobial resistance.

In the present study, the effect of cranberry extract on the prevention of bacterial adhesion was evaluated *in vitro*. Urine samples were collected from dogs receiving cranberry extract and used to determine antibacterial effects. Because *E coli* are the most common uropathogenic bacteria in dogs with UTIs,²⁻⁴ those bacteria were used in the present study. Three *E coli* strains were prepared for use in bacteriostasis and antiadhesion assays and microscopic examination. The bacteriostasis assay revealed no inhibition zone

around the urine samples and negative control sample, whereas the positive control sample (enrofloxacin) had an antibacterial effect (diameter of inhibition zone > 30 mm). This indicated 2 possibilities: the concentration of bacteria was too high for the cranberry extract to inhibit growth, or the urine samples from dogs receiving cranberry extract had no bacteriostatic activity.

Results of previous studies¹²⁻¹⁵ as well as the present study suggest that cranberries do not have an effect on inhibition of bacterial growth. Instead, it is hypothesized that cranberries prevent UTIs by blocking adherence of bacteria to the uroepithelium.¹⁶⁻¹⁸ Evidence to support this hypothesis was obtained in an *in vitro* study¹¹ of fimbriated *E coli* present in the urine 2 hours after ingestion of cranberry extract. In fact, the mean absorbance for the 3 *E coli* strains cultured with MDCK cells and urine samples obtained at 30 and 60 days was significantly lower than that after culture with the urine sample obtained before onset of cranberry extract administration, which indicated that the urine samples collected after administration of the cranberry extract had an antiadhesion effect. One possible mechanism of action may be that cran-

berry compounds act as receptor analogues and bind to the fimbriae of *E coli*, which thus competitively inhibits their adhesion. It has been confirmed that *E coli* isolated from dogs with UTIs most commonly express type-1 fimbriae.¹⁹ Furthermore, the main mechanism of in vitro adherence to canine uroepithelial cells involves a mannose-sensitive mechanism.²⁰ Components of the cranberry extract also might have altered P-fimbriated uropathogenic bacteria in other ways, such as by reducing adhesion capabilities, reducing fimbrial length and density, or inducing other morphological changes.^{18,21,22}

Mean *E coli* adherence to MDCK cells after incubation with urine samples obtained at 30 and 60 days was significantly lower, compared with adherence after incubation with the urine sample obtained before administration of the cranberry extract. Moreover, mean *E coli* adherence was significantly lower after incubation with the urine sample obtained at 60 days, compared with results after incubation with the urine sample obtained at 30 days.

In the present study, MDCK cells were used because they are a good in vitro method of screening to detect bacteria virulence²³ or determining the pathogenesis of various bacterial infections, including those attributable to uropathogenic *E coli*.^{24,25} Adhesion of uropathogenic *E coli* to epithelial cells can lead to ascending UTIs, which range from nonclinical bacteriuria to cystitis and acute pyelonephritis to more severe acute lobar nephronia.²⁶ Bacterial adhesion to uroepithelial cells by fimbrial or nonfimbrial adhesins in bacterial renal infections is an important factor in the subsequent development of UTIs in the upper urinary tract (ie, calyx, renal pelvis, and ureter) via the ascending route.²⁷ The effect of cranberry extract on *E coli* adhesion to both kidney epithelial cells and uroepithelial cells derived from dogs has been described.²⁸ Results for the microscopic examination performed in the present study correlate with results of another study²⁸ that also revealed antiadhesion activity of cranberries or cranberry extract on *E coli* adherence to specific primary-cultured uroepithelial cells.²⁸ The antiadherence effect of cranberries is not restricted to a particular group of *E coli* strains, which might otherwise be caused by interference with specific receptor-ligand modes of bacterial adhesion or by inhibition of expression of the bacterial fimbriae.^{21,29} The effect of cranberry intake might be synergistic, but the details remain unclear. In the present study, we minimized the possible bias associated with a noncontrolled trial by developing a bioassay to test adhesion of bacteria to MDCK cells that were cultured with urine obtained from dogs after they had received cranberry extract.

Antimicrobial resistance is an increasing concern. Therefore, alternative strategies such as consumption of cranberries or cranberry extract may be an option for prevention of UTIs in dogs. The present study revealed that the efficacy of cranberry extract for the prevention of UTIs was almost the same as that for an

antimicrobial (cephalexin), with a lower risk of antimicrobial resistance or superinfection.³⁰ Analysis of the results of the study reported here indicated that cranberry extract decreased *E coli* adherence to MDCK cells but did not inhibit bacterial growth. This effect suggested that cranberry extract has a potential clinical benefit for the prevention of UTIs in dogs.

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Footnotes

- a. Keflex, 250-mg capsule, Taiwan Biotech Co, Taoyuan City, Taiwan.
- b. 120 g/pack, Cranimals, West Vancouver, BC, Canada.
- c. Millilex GV, Millipore Ireland BV, Carrigtwohill Co, Cork, Ireland.
- d. Difco, Becton Dickinson and Co, Franklin Lakes, NJ.
- e. BBL, Becton Dickinson and Co, Franklin Lakes, NJ.
- f. Test disc, Oxoid, Basingstoke, Hampshire, England.
- g. Mediatech Inc, Tewksbury, Mass.
- h. Sunrise Infinite F200, Tecan, Männedorf, Switzerland.
- i. Magellan, version 6.6, Tecan, Männedorf, Switzerland.

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