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Adherence of uropathogenic *Escherichia coli* in dog urine after consumption of food supplemented with cranberry (*Vaccinium macrocarpon*)

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Abstract

Introduction: *Escherichia coli* is the most common pathogen isolated from the urine of dogs with urinary tract infections (UTIs). While there are many studies in humans investigating the potential for the prevention of UTIs by dietary consumption of cranberry, few analogous studies have been carried out in dogs. **Material and Methods:** Eight dogs, four male and four female, were successively fed two diets, first a control without cranberry, and then the second diet containing cranberry extracts. Naturally excreted urine was collected on the tenth day after the start of each diet for 24 h and used for bacterial growth. Madin-Darby canine kidney cell adherence by the uropathogenic *E. coli* G1473 strain expressing type 1 pili and positive for P pili and haemolysin gene markers was quantified after growth in urine samples. **Results:** Significant reductions in bacterial adherence to MDCK cells (from -16.5 to -73.4%, $P < 0.05$) were observed in the four females but not in the males after consumption of the cranberry extracts compared to the same animals consuming the control diet. **Conclusion:** Dietary supplementation with cranberry may provide some degree of protection to female dogs against adhesion of uropathogenic *E. coli* to urinary epithelial cells.

Keywords: dog, urinary tract infection, cranberry, *Escherichia coli*, adherence.

Introduction

Ascending urinary tract infections (UTIs) in dogs are common, *Escherichia coli* being the most common uropathogenic agent (17, 24). Uropathogenic *E. coli* (UPEC) strains predominantly belong to the phylogenetic groups B2 and D (25). They possess a battery of virulence factors to resist immune defences (capsule), induce cellular and tissue alterations (haemolysin and cytotoxic necrotising factors), optimise growth by avoiding iron deficiency (siderophores), adhere to epithelial cells and invade them (type 1 fimbriae, pyelonephritis-associated pili and S fimbriae) (24, 25). Various risk factors for the development of UTIs have been identified in dogs. These include gender, age, (a higher incidence being noted in older females), certain medical procedures or treatments such as bladder catheterisation, and certain diseases such as hyperadrenocorticism or diabetes

mellitus (2, 5, 7, 16). These infections are divided into simple uncomplicated infections, which are usually cystitis occurring in healthy subjects without urinary tract abnormalities, and complicated or recurrent infections occurring mainly in subjects with comorbidities or urogenital tract abnormalities. Complicated and recurrent UTIs are more difficult to treat and may be associated with therapeutic failure and the development of antimicrobial resistance (27). In general, the use of antibiotics in the treatment of urinary tract infections is associated with an increased incidence of antibiotic resistance in dogs (3). This situation highlights the need for alternative therapeutic solutions to antibiotic therapy for treating urinary tract infections in dogs (19).

The ascending colonisation of the urinary tract by UPEC strains linked to their pili-mediated adhesion to urinary epithelial cells plays a central role in bacterial persistence and the development of UTIs (20, 25). Two

major adhesion factors play an essential role in UTIs: type 1 fimbriae which have D-mannose derivatives as receptors and pyelonephritis-associated pili, also called P pili, which bind to Gal(1-4)-Gal oligosaccharide receptors (23). Numerous studies have shown the efficacy of cranberry extracts used in experiments *in vitro* and *ex vivo* in reducing the adhesion of UPEC strains to human urinary epithelial cells (8). *In vivo* studies have shown a tendency to reduce bacteriuria and prevent urinary tract infections in women (8). The bioactive molecules in cranberry are proanthocyanidins which act by inhibiting the adhesion of P pili to their cell receptor.

Very few equivalent studies exist in dogs. Cranberry extracts used in the experiments *in vitro*, either during bacterial growth or during bacteria-epithelial cell interaction, inhibited the adhesion of UPEC strains to the Madin-Darby canine kidney epithelial cell line (MDCK) (4, 15). However, only one published study has shown this anti-adhesion activity *ex vivo* (4). The present study was conducted to evaluate the effect of dietary cranberry supplementation on the *in vitro* adhesion of *E. coli* to canine uroepithelial cells in the urine of dogs in good general health.

Material and Methods

Animals. A total of 8 beagles in good general health (based on serum and urine biochemistry, urine cytology and urine microbiology), comprising four males (dogs 1 to 4) and 4 females (dogs 5 to 8) at one year of age, participated in this study. The weight of dogs 1 to 8 was 13.3, 13.4, 11.4, 10.6, 12.5, 10.9, 9.7, and 9.7 kg, respectively. Each dog was successively fed two diets: first the control diet, and next the same diet supplemented with 0.2% cranberry (*Vaccinium macrocarpon*) fruit powder (20 ppm proanthocyanidins “as fed”), the other ingredients being exactly the same. The percentage of inclusion in the diet was defined based on the estimated minimum dose found to be effective in dogs by Chou *et al.* (4). The average daily dose of proanthocyanidins per dog was 5 mg. The dogs were fed their usual quantities in order to maintain their body weight (130 kcal metabolisable energy/kg optimal weight^{0.75}). Tap water was offered *ad libitum*. Naturally voided urine was collected on the tenth day after the start of each diet for 24 h. Urine samples designated U1–8 corresponding to dogs 1–8 fed the control diet and UCR1–8 corresponding to the same dogs fed the supplemented diet were placed at 4°C immediately after collection and then stored at –20°C for later analysis. The dogs were housed in groups during the whole trial, except for the 24 h of urine collection, during which they were placed in metabolic cages adapted to their size. All experimental procedures were approved by the ethics committee of the Pays de la Loire (project referenced under the number

APAFIS#27382-2020092821441577 v6), and authorised by the French Ministry of Education and Scientific Research (notification January 4, 2021).

Adherence to canine uroepithelial cells. Urine samples were thawed at 4°C, centrifuged at 4,000 × g for 15 min and sterilised by filtration (0.45 µm). Bacterial adherence experiments were carried out *in vitro* with the canine uroepithelial MDCK cell line (ATCC CCL-34, which are cells derived from the normal kidney tissue of an adult female cocker spaniel) and with the UPEC strain G1473 (expressing type 1 pili; positive for the *papC* gene marker for P fimbriae and for the *hlyA* alpha haemolysin gene) as previously described (6). Monolayers of the MDCK cell line were grown at 37°C for 48 h on 24-well tissue culture plates (Corning, part of Fisher Scientific, Illkirch, France) in Eagle’s minimum essential medium (EMEM; Merck, Molsheim, France) containing 10% foetal calf serum (v/v), and antibiotics (200 U of penicillin and 50 mg of streptomycin per litre). Before adhesion tests, cells were washed with phosphate-buffered saline (PBS; pH 7.2). Bacteria grown for 42 h in dog urine containing 5% (v/v) Luria Bertani broth (Difco, Le Pont de Claix, France) were harvested by centrifugation, suspended at 10⁸ bacteria per mL in EMEM, added to the tissue culture, and incubated for 3 h at 37°C. After extensive washes with PBS, cells were fixed in ethanol, stained with 30% Giemsa stain (v/v) (Sigma Aldrich, Saint-Quentin-Fallavier, France), and microscopically examined under oil immersion. An adhesion index representing the average number of bacteria per cell was determined by examining 100 cells. Each adhesion experiment was performed in duplicate and four independent experiments were carried out. The statistical significance of the differences was evaluated with the equal-variance Student’s *t*-test, following the variance test with Fisher’s *F* statistics. P-values below 0.05 were considered significant.

Results

Urine samples were used for the growth of *E. coli* prior to adhesion testing. Different levels of adherence to MDCK cells of the UPEC strain used were observed depending on the dogs and the diets (Table 1). An illustration of the G1473 strain adherence to MDCK cells is presented in Figure 1.

A large number of adhered bacteria were observed on the surface of MDCK cells with the bacteria grown in urine samples U1–U8 (Fig. 1B). A lower number of adhered bacteria was observed on these cells when they were grown urine samples UCR1–UCR8, the samples taken after the dogs were treated with cranberry powder (Fig. 1C). The means of the adherence indices obtained with bacteria grown in urine samples collected after the consumption of one dietary regimen and after consumption of the other regimen are presented in Table 1.

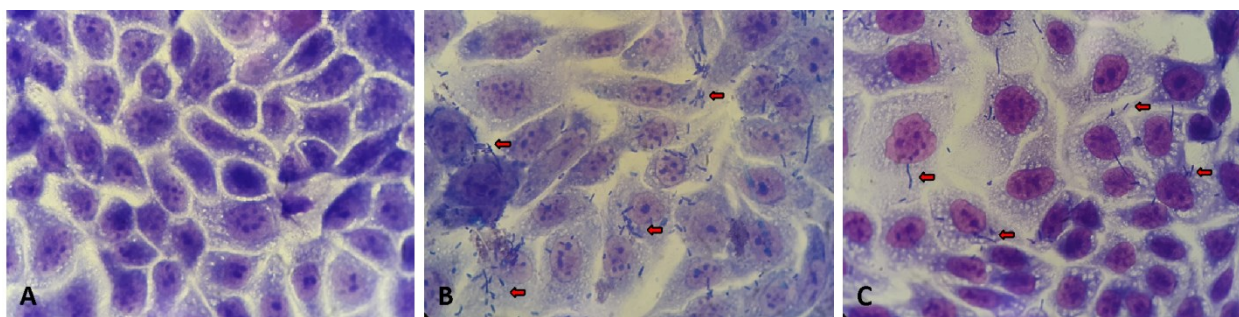


Fig. 1. Anti-adherence effect of cranberry extract consumption on the UPEC adherence to MDCK cells. Micrographs of Giemsa-stained preparations showing the UPEC G1473 strain adhering to MDCK cells after growth in urine samples

A – uninfected MDCK cells; B – UPEC adherence to MDCK cells after growth in urine sample U8; C – UPEC adherence to MDCK cells after growth in UCR8 urine sample. Red arrows: adhered bacteria

Table 1. Adherence indices of the G1473 UPEC strain to the MDCK cell-line

Sex	Dogs	Urine samples	Mean	SD	<i>P</i> Student's <i>t</i> test
Male	1	U1	4.22	2.91	0.055
		UCR1	5.78	4.42	
	2	U2	4.5	1.54	0.51
		UCR2	5.02	3.53	
	3	U3	3.07	0.11	0.57
		UCR3	3.55	1.76	
	4	U4	3.25	1.06	0.009
		UCR4	3.92	2.04	
Female	5	U5	7.89	4.33	1.34 10 ⁻³⁷
		UCR5	2.10	1.62	
	6	U6	6.75	3.45	4.97 10 ⁻¹⁷
		UCR6	3.38	2.05	
	7	U7	11.04	7.89	1.67 10 ⁻²⁴
		UCR7	4.68	3.99	
	8	U8	5.47	2.75	0.028
		UCR8	4.57	2.43	
Male + Female	1 to 8	U Total	5.65	2.39	1.1 10 ⁻³⁹
		UCR Total	3.91	0.95	

The results presented are the means of three independent experiments

U1 to U8 – urine samples from each of the eight dogs collected after consumption of the control diet; UCR1 to UCR8 – urine samples from each of the eight dogs collected after consumption of the control diet + cranberry extract supplement; U Total – value for the eight dogs after consumption of the control diet; UCR Total – value for the eight dogs after consumption of the control diet + cranberry extract supplement. *P*-values below 0.05 were considered significant. SD – standard deviation

Variations in the adhesion index of the strain G1473 after growth in the urine samples of individual dogs were observed for both diets. The bacterial adhesion index to MDCK cells ranged from 3.07 to 11.04 bacteria per cell after consumption of the control food and ranged from 2.10 to 5.78 after consumption of the cranberry supplement, depending on the dog. The highest adherence index (11.04 bacteria per MDCK cell) was obtained with bacteria grown in urine sample U7 collected from dog 7 after consumption of the control diet (Table 1). The lowest adherence index (2.10 bacteria per MDCK cell) was obtained with bacteria grown in urine sample UCR5 collected from

dog 5 after consumption of the control diet with cranberry extract. The mean of the adhesion indices obtained after the growth of bacteria in the urine of the eight dogs after consumption of the control diet was 5.65 ± 2.39 bacteria per MDCK cell. The mean of the adhesion indices obtained under these conditions for the four male (U1 to U4) and four female dogs (U5 to U8) were 3.82 ± 0.65 , and 7.48 ± 1.99 , respectively. Adhesion was significantly higher after growth in the urine of unsupplemented females than in that of unsupplemented males ($P < 0.001$). The mean of the adherence indices obtained after UPEC growth in the urine of the eight dogs after benefitting from

supplementation was significantly decreased to 3.91 ± 0.95 ($P < 0.001$). Looking at the effect of cranberry consumption on a dog-by-dog basis, there was a significant decrease in the adhesion index for female dogs 5, 6, 7, and 8. The percentage decrease in adhesion after consumption of cranberry extract was 73.4% for dog 5, 49.9% for dog 6, 57.6% for dog 7, and 16.5% for dog 8. The mean decrease was $49.35 \pm 23.99\%$ for these four dogs.

Discussion

In this study, the human UPEC strain G1473 was used, which had previously been shown to adhere to human T24 and dog MDCK urinary epithelial cell-lines (6, 13). Incubation in dog urine prior to infection of MDCK cells confirmed the ability of this strain to effectively adhere to canine urinary epithelial cells in an *ex vivo* test. The adhesion indices of strain G1473 after incubation in dog urine were equivalent to the adhesion indices we previously obtained after incubation in human urine (6). This suggests a similar behaviour of this UPEC strain after growth in dog and human urine. The ability to adhere to both human and dog epithelial cells illustrates the cross-species transmission of some UPEC strains. Indeed, certain clones of extraintestinal pathogenic *E. coli* can colonise the intestinal and urinary tract of humans and pet dogs living in the same household and trigger a urinary tract infection in some cases (9, 10, 11).

The results obtained during this *ex vivo* study showed a significant effect of cranberry consumption in reducing the adhesion of *E. coli* to the MDCK cell line. This inhibitory effect corresponded on average to a decrease of approximately 50% in the number of bacteria adhering to epithelial cells. There is a difference in individual efficacy, as this inhibition reached more than 70% of the adhesion index for one female dog and 16.5% for another, and it was only observed in females. In similar studies in humans, a partial decrease in *E. coli* adherence to human uroepithelial cells was previously observed and ranged from 45 to 57% depending on the dose of cranberry and the particular study (7, 22). The results of the only published *ex vivo* study in dogs cannot be quantitatively compared with the results of these studies because the adherence protocols were very different (4). That study by Chou *et al.* (4) used methanol-fixed dog epithelial cells rather than live cells, and urine was only supplied with Dulbecco's modified Eagle's medium during the bacteria-cell interaction. Nevertheless, the authors clearly showed a reduction in the adherence of uropathogenic *E. coli* to MDCK cells in the presence of urine from dogs after the consumption of cranberry extracts. What is intriguing about our results is the lack of effect of cranberry consumption on male dogs. The adhesion was significantly higher after growth in the urine of the females than in that of the males fed the

control diet. Therefore, cranberry had an effect in animals with the highest bacterial adherence indices. The small number of animals in the two sex groups makes it difficult to establish whether there was a difference between the sexes or a simple coincidence explains the effect having been seen more pronouncedly in female dogs. In their publication, Chou *et al.* (4) did not present the adherence values for each dog, just the values for all six dogs, which were three neutered males and three neutered females. If they mixed the urine of the dogs, no difference between males and females would have been possible to see. In the same report, cranberry extracts were also added to the diet of dogs that had suffered at least three episodes of urinary tract infections in the previous year. During the following six months, none of these dogs developed a UTI. Unfortunately, all dogs in this part of the *in vivo* study were female. In a randomised controlled trial involving 94 animals (36 females and 58 males), oral administration of cranberry had no effect on *E. coli* bacteriuria in dogs after decompressive surgery for acute thoracolumbar intervertebral disc herniation (18). In the same study, the authors observed no difference between the administration of cranberry or placebos on the presence of anti-adherence activity in a haemagglutination test in the urine of dogs. Another recent study showed a decrease in protein, epithelial cells, leukocytes, and bacterial counts in the urine of 10 dogs with bacterial cystitis 60 days after the start of oral cranberry administration (1). Unfortunately, the sex of the recruited dogs was not documented and the study did not include a control group of untreated dogs. Many studies have shown that cranberry consumption can reduce the incidence of recurrent urinary tract infections in women. However, the published studies are not unanimous and some studies have not shown the efficacy of cranberry in the prevention of female UTI, perhaps because of differences in protocols (subjects, clinical trial methods, cranberry products, doses administered, *etc.*) (8, 14). Only a few studies have shown the efficacy of dietary cranberry consumption in the prevention and treatment of UTI in men. These studies were limited to elderly subjects with prostatic hyperplasia. Dietary cranberry supplementation was associated with a significant decrease in episodes of UTI in men over 65 years of age with moderate hyperplasia (13). In a second study, a significant decrease in the international prostate symptom score was observed in cranberry recipients compared to placebo recipients in men over 45 years old, with a dose effect (26). In a rat model of chronic bacterial prostatitis, pre-treatment with cranberry decreased the degree of inflammation in prostate tissue compared to control rats (12). However, there was no significant decrease in the rate of infection or bacterial load in the treated group compared to the control group. The anti-inflammatory effect of cranberry on prostatitis is thought to be related to the flavonoid contents and in particular to quercetin (20, 21).

In conclusion, cranberry supplementation may provide a degree of protection for female dogs against the adherence of uropathogenic *E. coli* to urinary epithelial cells. Further experiments are required to understand the difference in the efficacy of cranberry in reducing *E. coli* adherence between females and males and to confirm this efficacy *in vivo* in the prevention and treatment of UTIs in dogs.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: The experimental dogs were housed at the Nantes Atlantic National College of Veterinary Medicine, Food Science and Engineering (ONIRIS) (Nantes, France), and the protocol implemented within the framework of the present study was carried out in accordance with the texts of the EU Directive (2010/63/EU) and the French Decree (no. 2013 118) on the protection of animals used for scientific, regulatory or educational purposes. All experimental procedures have been approved by the Ethics Committee of the Pays de la Loire (project referenced under the number APAFIS#27382-2020092821441577 v6), and authorised by the French Ministry of Education and Scientific Research (notification January 4, 2021).

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