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The effect of consumption of cranberry *(Vaccinium macrocarpon)* on *Escherichia coli* adherence to feline uroepithelial cells in a blind randomised cross-over trial in cats

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Abstract

Introduction: *Escherichia coli* is the most common uropathogen in humans, dogs and cats. Dietary consumption of cranberry (*Vaccinium macrocarpon*) is known to be associated with a reduction in uropathogenic *E. coli* (UPEC) adhesion to human and canine urinary epithelial cell lines, but this has not been shown in cats. **Material and Methods:** Six neutered domestic cats, one male and five females, were randomly fed three diets successively, one containing 0.1% cranberry powder, one containing 0.3% cranberry powder, and one being the control without cranberry. Naturally emitted urine was collected on the last two days of each period of two weeks and used for bacterial growth. Adherence to Crandell–Rees feline kidney (CRFK) uroepithelial cells of the feline UPEC C571 strain (positive for the *pap*C gene marker for P-fimbriae and the *fim*A marker for type 1 pili and negative for the gene of the alpha haemolysin cytotoxin *hly*A, and additionally non-haemolytic *in vitro* on blood agar) was quantified after growth in urine samples. **Results:** Significant reductions in bacterial adherence to CRFK cells were observed for 60% of cats receiving 0.1% cranberry powder supplementation and for all cats receiving 0.3% cranberry powder supplementation, compared to the same animals consuming the control diet. **Conclusion:** Dietary supplementation with cranberry may provide some degree of protection to cats against adhesion of UPEC to feline uroepithelial cells.

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

Introduction

Urinary tract infections (UTIs) can be defined as the persistence of an infectious agent in large numbers within the urogenital tract, causing an inflammatory response and clinical signs. The clinical aspects of UTIs in cats were reviewed and updated by Dorsch *et al.* (10) recently. The prevalence of UTIs in cats is relatively low, and many cats only manifest asymptomatic bacteriuria, which is called subclinical bacteriuria, but the incidence of UTIs in the species increases with age and the administration of treatments or presence of other pathologies (3, 13, 17). Whether symptomatic or asymptomatic, UTIs in cats are associated with an increase in the concentration of erythrocytes and leukocytes in the urine (17). The most common uropathogenic bacteria in cats are *Escherichia coli*, *Enterococcus* spp. and *Staphylococcus* spp. (16, 21), uropathogenic *E. coli* (UPEC) being the most frequently observed of these. Despite geographic differences in the presence of virulence genes in feline UPEC and the absence of a specific genotype responsible for cystitis in cats, the vast majority of feline UPEC belong to the particularly virulent B2 phylogenetic group (11, 17, 24, 29). Thus, as with UPEC isolated from dogs or humans, many feline UPEC harbour the genes coding for type 1 and type P pili, which allows them to ascend the urinary tract and cause cystitis and pyelonephritis (15, 24, 29).

Once a UTI is diagnosed, promptly administered antimicrobial therapy usually results in clinical improvement and can reduce the severity of microbe-induced inflammatory kidney damage (30). Conventional oral antimicrobial therapy is effective against the majority of

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common UTIs, but is often ineffective against persistent or recurrent infections (11, 19). As recurrent UTIs are usually linked to the same strain of UPEC, ineffective antibiotic treatment can lead to the selection of antimicrobial resistance (1, 12). Nevertheless, most cat UPEC are sensitive to antimicrobials commonly used for veterinary treatment of UTIs, fewer than 10% of them being multi-resistant to antibiotics (2). Antibiotic resistance is, however, a growing problem in both human and veterinary medicine (25), which encourages the consideration of non-antimicrobial treatments as alternatives to antibiotic therapy for UTIs (26, 28).

Extracts of cranberry (*Vaccinium macrocarpon*) reduce the *in vitro* adhesion of UPEC to human, canine and feline urinary epithelial cells (20). Several *ex vivo* studies have shown that dietary supplementation with cranberry reduces the adhesion of UPEC to human and canine urinary epithelial cells in urine (4, 5, 9, 18, 27). A preliminary study carried out in cats suffering from feline idiopathic cystitis showed that dietary supplementation with cranberry led to a disappearance of clinical signs of a lower UTI after 60 days (6). In another study, dietary supplementation with a mixture of cranberry extract, glucosamine and parsley extract led to clinical improvement after 21 days for 62.5% of elderly cats with cystitis (22). It seems that the effectiveness of cranberry or its extracts is substantiated in cats, but so far, there is no evidence on the mode of its action in these animals. The aim of the study was to determine the effect of dietary cranberry supplementation on UPEC adhesion to feline urinary epithelial cells in an *ex vivo* study in cat urine. The hypothesis was that, as for humans and dogs, the use of cranberry may decrease the adhesion of UPEC to feline epithelial cells.

Material and Methods

Cats. Five female and one male neutered domestic short-haired cats at six years of age and with a mean body weight 5.3 ± 0.5 kg were included in the study. The cats were considered healthy based on biochemistry tests, urine analysis and veterinary physical examinations performed before the start of the study. During the whole trial, the cats' health was also monitored daily through caretakers' observations. For urine collection, cats were housed in individual rooms, with free access to a shared outdoor area through automatic doors activated by individual cats' microchips. Individual rooms and the outdoor area offered elements allowing the expression of natural behaviour and for behavioural needs to be met, such as scratching, hiding and climbing. All the cats were trained to eat, drink and urinate in their own room. Each individual room had its own litter box connected to a urine collection system for the individual cat's naturally voided urine with refrigerated storage at +4°C over a 24 h period. The urine collected during 24 h was then stored at −80°C until analysis.

A Latin square design (6 cats × 3 diets × 3 periods) was used with the cats randomised to test each diet in a random order in six possible sequences. Each period was two weeks long and one week's washout was allowed between periods. Urine was collected the last 2 days of each period. The control diet was a diet formulated for adult cats (% as fed: protein 37.2, fat 10.5, ashes 6.9, crude fibre 2.5 and moisture 4.5). The test diets were exactly the same as the control diet but supplemented with 0.1 or 0.3% cranberry powder (corresponding to 10 or 30 ppm proanthocyanidins (PAC), respectively). The cats were fed their usual quantities in order to maintain their body weight (61 g ± 10g or 76 ± 12 kcal metabolizable energy/kg body weight$^{\wedge 0.67}$). The average daily dose of PAC per cat was 0.61 ± 0.1 or 1.83 ± 0.3 mg/day (0.2 ± 0.03 or 0.6 ± 0.08mg /kg body weight$^{\wedge 0.67}$) according to the proportion of cranberry powder. The high dose corresponded to the inclusion level (expressed in mg PAC intake/kg metabolic body weight/day) that has been shown to be efficient in dogs' urine at reducing bacterial adherence to Madin–Darby canine kidney epithelial cells (4). Low-mineral-content water was offered *ad libitum*. The protocol was approved by the Affinity Petcare ethical committee and the appropriate organ in the Generalitat de Cataluña in Spain (9019/24958/201).

Adherence to feline uroepithelial cells. After thawing at +4°C, the urine samples were centrifuged at 4,000 × *g* and then sterilised by filtration (0.45 µm). The Crandell–Rees feline kidney (CRFK) uroepithelial cell line (ATCC CCL-94) and the UPEC C571 strain were used. This strain was selected because it is positive for the gene markers for P-fimbriae *pap*C and type 1 pili *fim*A, which are adhesins and denote virulence through adherence, and because the strain is negative for the gene of the alpha haemolysin cytotoxin *hlyA* and non-haemolytic *in vitro* on blood agar, which imply that the strain's pathogenicity was not in cytotoxicity but in adherence mechanisms alone. The feline kidney cells were grown on 24-well tissue culture plates until confluent. Bacteria were grown for 42 h in cat urine supplemented with 5% (v/v) Luria–Bertani broth. Bacterial suspensions calibrated at 10^8 bacteria per mL in Eagle's minimum essential medium obtained after centrifugation were added to confluent monolayers of cells and incubated for 3 h at 37°C. Non-adherent bacteria were removed by successive washes with phosphate-buffered saline and the bacteria and cells were fixed in ethanol and stained with 30% Giemsa (v/v). The average number of adherent bacteria per cell was determined by examining 100 cells, yielding the adhesion index. Four independent adhesion experiments were performed, with one duplicate for each experiment. The results were compared with Student's *t*-test with equal variance, after a variance test with Fisher's F statistics. Values of P less than 0.05 were considered significant.

Results

The level of adherence of the feline UPEC strain C571 to CRFK cells depended on the cat and diet (Table 1). Figure 1 shows the adhesion to CRFK cells of strain C571 grown in urine samples from cat No. 2 after consumption of the different diets. A large number of adherent bacteria were observed on the surface of CRFK cells when they were grown in urine samples collected after the provision of the control diet (Fig. 1B). A lower number of adherent bacteria were observed after consumption of the diet and cranberry supplement at 0.1% (Fig. 1C). The number of adherent bacteria fell further with the increase of the proportion of cranberry to 0.3% (Fig. 1D).

The means of the adherence indices obtained with bacteria grown in urine samples of the different cats collected after the consumption of the three diets are presented in Table 1. Cat No. 4 was removed from the study following the diagnosis of an allergy.

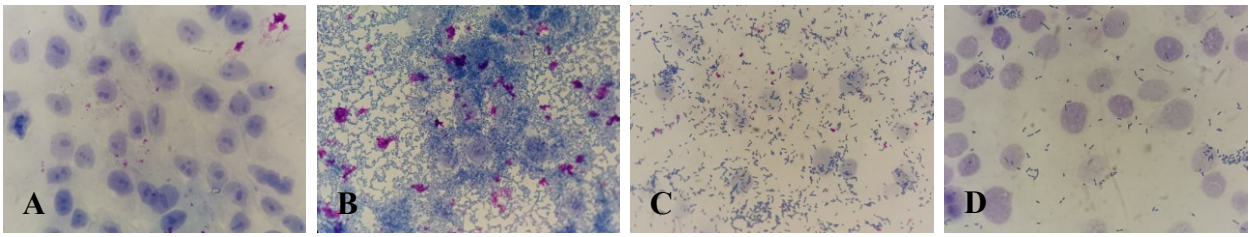

Fig. 1. Anti-adherence effect of cranberry extract consumption on uropathogenic *E. coli* (UPEC) adherence to cat urinary epithelial Crandell–Rees feline kidney (CRFK) cells. The effect is shown in micrographs of Giemsa-stained preparations in which feline UPEC strain C571 appears adhering to CRFK urinary epithelial cells after growth in urine samples. A – uninfected CRFK cells; B – UPEC adherence to CRFK cells after growth in urine sample U2 collected from cat 2 given the control diet; C – UPEC adherence to CRFK cells after growth in urine sample U2CR0.1 collected from cat 2 given cranberry powder at 0.1% in its diet; D – UPEC adherence to CRFK cells after growth in urine sample U2CR0.3 collected from cat 2 given cranberry powder at 0.3% in its diet

Table 1. Mean indices of the C571 uropathogenic *E. coli* strain's adherence to Crandell–Rees feline kidney uroepithelial cells

Cat No./Sex	Urine samples	Mean	SD	P-value Student's *t*-test
1/male	U1	0.69	0.48	
	U1CR0.1	0.77	0.49	$0.51^{\lozenge}$ (NS)
	U1CR0.3	0.59	0.59	$0.011^{\lozenge}$ (S) $0.044^{\lozenge\lozenge}$ (S)
2/female	U2	27.9	5.7	
	U2CR0.1	13.03	9.22	$2.67\ 10^{-36\lozenge}$ (S)
	U2CR0.3	2.35	2.00	$0.0002^{\lozenge}$ (S) $4.48\ 10^{-50\lozenge\lozenge}$ (S)
3/female	U3	3.16	2.85	
	U3CR0.1	0.93	0.26	$2.55\ 10^{-36\lozenge}$ (S)
	U3CR0.3	1.17	0.88	$3.86\ 10^{-22\lozenge}$ (S) $0.21^{\lozenge\lozenge}$ (NS)
4/female	U4	Removed		
	U4CR0.1			
	U4CR0.3			
5/female	U5	1.43	1.00	
	U5CR0.1	1.16	0.50	$0.18^{\lozenge}$ (NS)
	U5CR0.3	0.91	0.68	$0.005^{\lozenge}$ (S) $0.07^{\lozenge\lozenge}$ (NS)
6/female	U6	1.31	0.93	
	U6CR0.1	0.93	0.37	$8.55\ 10^{-7\lozenge}$ (S)
	U6CR0.3	1.17	0.23	$0.0007^{\lozenge}$ (S) $0.08^{\lozenge\lozenge}$ (NS)

The results are the means of four independent experiments. All six cats were neutered. U1 to U6 – samples of urine passed after the six cats had consumed the control diet; U1CR0.1 to U6CR0.1 – samples of urine passed after the six cats had consumed the control diet + cranberry powder at 0.1%; U1CR0.3 to U6CR0.3 – samples of urine passed after the six cats had consumed the control diet + cranberry powder at 0.3%. P-values < 0.05 were considered significant. S – significant (P-value < 0.05); NS – not significant (P-value ≥ 0.05); SD – standard deviation; ◊ – P-value *vs.* control diet; ◊◊ – P-value *vs.* control diet + cranberry powder at 0.1%. Cat No. 4 was withdrawn following the diagnosis of an allergy

The highest adherence index (27.9 bacteria per cell) was noted for bacteria grown in urine sample U2 collected from cat No. 2 after consumption of the control diet (Table 1). The lowest adherence index (0.59 bacteria per cell) was obtained with bacteria grown in urine sample U1CR0.3 collected from cat No. 1 after consumption of the control diet with cranberry powder at 0.3%. Variations in the adherence index of strain C571 after growth in the urine of individual cats were observed for the three diets. The bacterial adherence index to CRFK cells ranged from 0.69 to 27.9 bacteria per cell after consumption of the control food, from 0.77 to 13.03 after consumption of the cranberry powder at 0.1%, and from 0.59 to 2.35 after consumption of cranberry at 0.3%. The decrease in adherence varied by cat from 18.9 to 70.7% after consumption of cranberry powder at 0.1% and from 10.9 to 91.6% after consumption of it at 0.3%. For four of the five cats included in the study, urine samples taken after dietary supplementation with 0.1% cranberry powder significantly inhibited the adhesion of UPEC to CRFK cells (Table 1). For cat No. 1, no inhibition of UPEC adherence was observed at this concentration, but the adherence index was low after bacterial growth in the urine samples of this cat regardless of the diet. For all the cats studied, urine samples collected after dietary supplementation with 0.3% cranberry powder significantly inhibited UPEC adherence to CRFK cells compared to the samples collected after providing the control diet without cranberry. A significant dose effect, *i.e.* a significantly lower UPEC adherence to CRFK cells after 0.3% cranberry supplementation compared to 0.1% supplementation was observed for cats Nos 1 and 2.

Discussion

Some studies have shown that dietary cranberry supplementation may be beneficial for the treatment of lower UTIs in cats (6, 22). By analogy with *ex vivo* studies carried out in humans and dogs (4, 5, 9) and an *in vitro* study with the CRFK feline cell line (20), it was likely that cranberry also acted in cats by inhibiting the adherence of UPEC to feline urinary epithelial cells. In this study, dietary supplementation of cats with cranberry was found to be effective in decreasing UPEC adherence to feline CRFK cells in urine. A significant reduction in adherence to feline CRFK cells was achieved for 60% of the cats with the 0.1% cranberry powder supplementation and for all cats with the 0.3% cranberry powder supplementation. As previously demonstrated in dogs (4), individual variations in the magnitude of adherence decrease were observed in cats. The percentage of reduction in adherence varied by cat from 19 to 71% with an average of 34% with the 0.1% proportion of cranberry, and from 15 to 92% with an average of 43% with the 0.3% proportion. An inhibition of adhesion of approximately 50% had been observed in dogs and one varying from 45 to 57% in humans in equivalent *ex vivo* studies (4, 9, 27).

The anti-adherence activity of cranberry is linked to its effects on bacterial pili. A-type proanthocyanidins (PAC-A) are polyphenolic molecules present in high concentrations in cranberry that inhibit the attachment of bacterial pili to their cellular receptors by acting as receptor analogues (14). After ingestion, PAC are catabolised by colonic microflora, generating a diversity of phenolic acids which are absorbed into the circulatory system and excreted in urine, where they can inhibit bacterial adherence (7, 8). Cranberry can also induce changes in the expression of P pili by *E. coli*, reducing their density and length (18). In humans, a daily dose of 72 mg of PAC-A divided into two doses of 36 mg was effective in preventing UTIs (23). In published clinical studies evaluating the effectiveness of dietary supplementation with cranberry to prevent or treat urinary infections in cats, there was no calculation of the daily dose of PAC administered (6, 22). In this cat study, two average daily doses of PAC were used. They were 0.61 ± 0.1 and 1.83 ± 0.3 mg/day, *i.e.* 0.2 ± 0.03 or 0.6 ± 0.08 mg/kg body weight$^{\wedge 0.67}$. The highest dose corresponded to that shown to be effective in dog urine in reducing bacterial adhesion to MDCK cells (4). Cats, dogs and humans share some UPEC strains that have increased antibiotic resistance (2, 15, 25). Reducing the incidence of urinary tract infections through dietary cranberry supplementation in these three species would be beneficial in reducing the circulation of antibiotic-resistant *E. coli* strains in both veterinary and human medicine.

Conclusion

As it does for dogs and humans, cranberry supplementation may provide a degree of protection for cats against the adherence of UPEC to urinary epithelial cells. Both dosages used in this study (0.2 ± 0.03 and 0.6 ± 0.08 mg/kg body weight$^{\wedge 0.67}$) may be effective in reducing bacterial adherence to urinary epithelial cells in cats, a stronger and more consistent effect to be expected at the higher dose. However, these doses must be confirmed during clinical studies of dietary supplementation with PAC for the prevention and treatment of urinary infections in cats. Furthermore, as this blind randomised cross-over trial involved a relatively small number of cats, its results will need to be confirmed in the future by a study based on a larger number of animals.

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Animal Rights Statement: The protocol implemented in the present study was carried out in accordance with EU Directive (2010/63/EU) on the protection of animals used for scientific, regulatory or educational purposes.

All experimental procedures took place at Affinity Petcare (Lleida, Spain) and were approved by the Affinity Petcare ethical committee and the appropriate organ in the Generalitat de Cataluña (9019/24958/201).

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