Prevalence of urovirulence genes *cnf*, *hlyD*, *sfa/foc*, and *papGIII* in fecal *Escherichia coli* from healthy dogs and their owners

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Objective—To determine the prevalence of 4 urovirulence genes in fecal *Escherichia coli* isolates from healthy dogs and their owners and to determine whether detection of *E coli* strains with these genes was associated with a history of urinary tract infection (UTI).

Sample Population—61 healthy dog-owner pairs and 30 healthy non–dog owners.

Procedures—A fecal specimen was obtained from each participant, and 3 colonies of *E coli* were isolated from each specimen. A multiplex PCR assay was used to detect 4 genes encoding virulence factors: cytotoxic necrotizing factor (*cnf*), hemolysin (*hlyD*), s-fimbrial and F1C fimbriae adhesin (*sfa/foc*), and pilus associated with pyelonephritis G allele III (*papGIII*). Human participants completed a questionnaire to provide general information and any history of UTI for themselves and, when applicable, their dog.

Results—26% (16/61) of dogs, 18% (11/61) of owners, and 20% (6/30) of non–dog owners had positive test results for ≥ 1 *E coli* virulence gene. One or more genes were identified in fecal *E coli* isolates of both dog and owner in 2% (1/61) of households. There was no difference in the detection of any virulence factor between dog-owner pairs. Female owner history of UTI was associated with detection of each virulence factor in *E coli* strains isolated from their dogs' feces.

Conclusions and Clinical Relevance—Dogs and humans harbored fecal *E coli* strains possessing the genes *cnf*, *hlyD*, *sfa/foc*, and *papGIII* that encode urovirulence factors. It was rare for both dog and owner to have fecal *E coli* strains with these virulence genes. (*Am J Vet Res* 2009;70:1401–1406)

An estimated $1.6 billion is spent annually on community-acquired UTIs in humans.[1] *Escherichia coli* is the most prevalent bacterial species isolated from urine in humans and dogs with UTIs. This potential pathogen is responsible for 85% to 95% of cases of uncomplicated cystitis, > 90% of cases of uncomplicated pyelonephritis in women,[2] and 44% of all UTIs in dogs.[3]

Most UTIs are believed to originate from the affected individual's own flora, and there are 2 theories to explain this mode of infection. The prevalence theory suggests that bacterial strains with highest prevalence in the GI tract and perineum will travel horizontally across the urogenital mucosa and ascend the distal urethra, whereas the pathogenicity theory suggests that only strains possessing certain virulence traits giving the microorganisms enhanced ability to travel, colonize, and invade the urinary tract will succeed.[4,5] Molecular and epidemiologic studies of uropathogenic *E coli* isolates have led to additional hypotheses about the original sources of these strains, suggesting the possibility that uropathogenic *E coli* may be transmitted from animals to humans, either from livestock through the food chain or through contact with companion animals.

In 2001, a single clonal group of multidrug-resistant uropathogenic *E coli*, named clonal group A, was responsible for nearly half of community-acquired UTIs in women in multiple US states.[6] The same strain was isolated from the feces of healthy human volunteers

Abbreviations

cnf	Cytotoxic necrotizing factor gene
hlyD	Hemolysin gene
MUG	4-Methylumbelliferyl-β-D-glucuronide
papGIII	Pilus associated with pyelonephritis gene G allele III
sfa/foc	S-fimbrial adhesin and F1C fimbriae gene
UTI	Urinary tract infection

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during the same period.[6] One explanation was an outbreak from dissemination of contaminated food. In an epidemiologic study,[7] investigators compared 495 animal and environmental *E coli* isolates with clonal group A isolates and found that 26% had similar molecular fingerprints when an ERIC2 PCR assay was performed, but only 1 animal isolate (from a cow) had a similar fingerprint when pulsed-field gel electrophoresis was used instead. No animal isolates in that study had virulence factor patterns or antimicrobial susceptibility patterns identical to those of human clonal group A isolates. The public-health implications of multidrug-resistant uropathogenic *E coli* being spread by contaminated food products would be considerable if this actually did take place.

Dogs have also been considered a possible reservoir for uropathogenic *E coli* on the basis of studies[8–11] in which genes for similar urovirulence factors were identified, including *cnf*, *hlyD*, *sfa/foc*, and *papGIII* in *E coli* isolated from the feces and urine of dogs and women. These studies revealed similar virulence factors in *E coli* isolates from dogs and women that did not have direct or known shared environmental contact. It is hypothesized that if dogs are indeed important reservoirs of uropathogenic *E coli*, then that *E coli* isolated from the feces of dogs and owners living within the same household would have similar virulence factors.

The purpose of the study reported here was to determine the prevalence of 4 urovirulence genes (*cnf*, *hlyD*, *sfa/foc*, and *papGIII*) in *E coli* recovered from the feces of healthy dogs and their owners and to determine whether *E coli* isolates with these genes were associated with a history of UTI in either species.

Materials and Methods

Participants—Dog-owner pairs and non–dog owners were recruited from among the faculty, staff, and students of the College of Veterinary Medicine and other colleges within the University of Tennessee as well as from among employees of private local business. All human participants were required to be healthy and > 18 years of age. All canine participants were considered healthy by their owners. All human and canine participants were required not to have diarrhea at the time of the study. For dog-owner pairs, dogs and their owners were required to live in the same household for at least 6 months, and only 1 dog and owner/household could participate. Non–dog owners were required not to own a dog or cat and to have no more than 1 hour of dog contact/wk. All human and canine participants were required not to have received antimicrobial treatment within the 2 weeks preceding enrollment. A sample size calculation indicated that 61 dog-owner pairs would be needed to achieve 98% power by use of a McNemar χ^2 analysis, with an effect size of 0.30 and an α value of 0.05. The study design was approved by the Institutional Review Board and Institutional Animal Care and Use Committee of the University of Tennessee to ensure appropriate treatment of all participants. Consent for participation was obtained from all human participants.

Fecal specimen collection—To obtain fecal specimens, 1 swab collection system containing transport medium[a] was provided for each participant. Owners were instructed to collect a fecal specimen from themself and their dog within the same 12-hour period and submit the specimens together. Specimens were labeled, double-packaged, refrigerated, and submitted by human participants directly to the primary investigator within 24 hours after collection. Each human participant was asked to answer a questionnaire to obtain dog (if relevant) and human information and any history of UTI. Reporting of UTIs was dependent on participant recall; no medical records were reviewed, and information regarding the manner in which the diagnosis of UTI was made was not obtained.

Isolation of *E coli*—Each fecal swab specimen was submerged in buffered peptone water, and 20 µL of the resulting solution was streaked onto each of 2 culture plates containing eosin methylene blue agar with 0.1 g of MUG fluorescence crystals/L[b] or *E coli* agar with MUG.[c] The MUG was used to identify presumptive *E coli* colonies because most strains of *E coli* possess β-glucuronidase, which hydrolyzes MUG to 4-methylumbelliferone, causing colonies to fluoresce blue under long-wavelength (366 nm) UV light.[12] Inoculated plates were incubated at 44°C for 18 to 24 hours, then examined under UV light for evidence of fluorescence. Three MUG-positive colonies/plate, ideally those with different morphologic features, were selected, and each was used to inoculate a separate tube of brain heart infusion broth (5 mL). Tubes were incubated at 44°C for 18 to 24 hours. After incubation, 20 µL of broth was streaked onto plates of *E coli* agar with MUG[c] for isolation of *E coli*. Plates were incubated at 44°C for 18 to 24 hours, then examined under UV light. Identities of presumptive *E coli* colonies were confirmed with a biochemical test kit.[d] Confirmed, pure isolates of *E coli* were stored in brain heart infusion broth with 10% glycerol at –85°C until analyzed for virulence genes.

Multiplex PCR assay of virulence factors—In preparation for the PCR assay, 3 frozen *E coli* isolates/participant were grown on blood agar overnight at 35°C. Presence or absence of hemolysis was determined by examining the agar plates for large zones of clearing around bacterial colonies. One colony/isolate was transferred to a microscope slide, Gram stained, and examined with a light microscope to confirm that it contained a pure isolate of gram-negative bacilli. Bacteria from the same colony were then transferred to Luria-Bertani broth[e] and incubated overnight at 35°C. In attempt to remove inhibitors such as bile salts, polysaccharides, and hemoglobin, 1 mL of inoculated broth was centrifuged for 1 minute at 8,000 × *g* at 20°C, the supernatant was discarded, and the resulting pellet was suspended in 200 µL of sterile nuclease-free water. Suspensions were boiled at 100°C for 5 minutes, mixed with a vortex machine to disrupt cell membranes, and boiled for another 5 minutes. After boiling, suspensions were centrifuged for 1 minute at 8,000 × *g* at 20°C and the lysate supernatant was separated. Spectrophotometry[f] was used to confirm that extracted *E coli* lysates had sufficient DNA for the PCR assay based on an arbitrary cutoff of > 400 ng/µL (actual range, 523 to 2,063 ng/µL). Overall lysate quality was considered pure with

a mean 260:280-nm absorbance ratio of 1.81. The DNA lysates were stored at −85°C until the PCR assay was performed.

A multiplex PCR assay was used to detect genes *cnf*, *hlyD*, *sfa/foc*, and *papGIII* in DNA lysate. Sequences of primers (5′ to 3′) were as follows: *cnf* (974 bp) forward, atcttatactggatgggatcatcttgg; *cnf* reverse, gcagaacgacgttcttcataagtatc; *hlyD* (904 bp) forward, ctccggtacgtgaaaaggac; *hlyD* reverse, gccctgattactgaagcctg; *sfa/foc* (410 bp) forward, ctccggagaactgggtgcatcttac; *sfa/foc* reverse, cggaggagtaattacaaacctggca; *papGIII* (258 bp) forward, ggcctgcaatggatttacctgg; and *papGIII* reverse, ccaccaaatgaccatgccagac.[5,13] *Escherichia coli* strain J96[g] was used as a positive control sample for all 4 virulence genes, and *E coli* strain JJ055[g] was used as a negative control sample.[13] Nuclease-free water was also used as a negative control sample.

A master mix was prepared under a biosafety hood to minimize contamination and included the following (per reaction): 2.5 µL of 10X buffer,[h] 4 µL of 25mM $MgCl_2$,[h] 0.25 µL of polymerase[i] at a concentration of 5 U/µL, 2 µL of 2.5mM mixed deoxyribonucleotide triphosphates, 0.075 µL of 200µM of each primer, and 13.65 µL of sterile nuclease-free water. Plate[j] wells were loaded under a biosafety hood with 23 µL of master mix, followed by 2 µL of DNA lysate. Positive and negative control samples were included in each plate, and loaded plates were sealed with microseal film.[k] Each DNA sample was evaluated in triplicate to ensure accuracy.

A thermal cycler[l] was used for the PCR assay. Activation was performed at 95°C for 12 minutes. Twenty-five cycles of denaturation (94°C for 30 seconds), annealing (63°C for 30 seconds), and extension (68°C for 3 minutes) were performed. A final extension was performed at 72°C for 10 minutes. Twenty-five microliters of each PCR-assay product was analyzed by use of electrophoresis with a 2% agarose gel. Five microliters of loading dye[m] was added to each 25-µL PCR-assay product prior to loading. A 100-bp DNA ladder[n] was used as a standard to control the size of the amplified product, and 1.5 µL of loading dye[m] was added to 7.5 µL of DNA ladder[n] prior to gel loading. Positive and negative control samples were included on each gel. Gels were run at 150 V for 3 hours, with 1X tris-acetate EDTA buffer. Afterward, gels were stained with ethidium bromide (1 µL/500 mL of water) for 15 minutes, destained with water for 15 minutes, and photographed. A commercial software program[o] was used to analyze band size on the electrophoretic gel and to verify the presence or absence of virulence factor genes.

Statistical analysis—A commercial statistical software program[p] was used to compute all statistics. McNemar (paired data) and Pearson (unpaired data) χ^2 tests were used to analyze dichotomous data, such as association between presence of virulence genes in fecal *E coli* and history of UTI. The original threshold for significance was set at a value of $P \leq 0.05$. A Bonferroni adjustment was subsequently made for multiple comparisons; the adjusted value of *P* was calculated as the original value divided by the number of virulence genes (4), and a value of $P < 0.013$ was considered significant. The Cohen κ statistic was used to compare agreement between virulence factors (ie, whether the presence of 1 factor was associated with the presence of another factor). A κ value of 1.0 indicated perfect agreement, and a κ value of 0.8 to 1.0 indicated strong agreement.[14]

Results

Participants—Sixty-one dog-owner pairs and 30 non–dog owners were recruited. Overall, 74% (n = 45) of dogs, 82% (50) of dog owners, and 80% (24) of non–dog owners had negative PCR-assay results for all 4 virulence genes in all 3 *E coli* isolates obtained from their feces. Twenty-six percent (n = 16) of dogs, 18% (11) of dog owners, and 20% (6) of non–dog owners had positive test results for ≥ 1 *E coli* virulence gene (**Figure 1**). Ten percent (n = 6) of dogs, 7% (4) of dog owners, and 0 non–dog owners had positive PCR-assay results for all 4 virulence genes.

Virulence factor patterns—In fecal specimens from 93% (142/152) of participants, all 3 *E coli* isolates shared the same virulence gene pattern. The most common pattern was a negative PCR-assay result for all 4 genes. A positive PCR-assay result for all 4 genes was detected in 12% (n = 7) of dogs, 8% (5) of dog owners, and 0 non–dog owners. The combination of genes *cnf*, *hlyD*, and *sfa/foc* was detected in isolates from 3% (n = 2) of dogs, 8% (5) of dog owners, and 7% (2) of non–dog owners. The only virulence genes detected in the absence of all other genes were *sfa/foc* in isolates from 10% (n = 6) of dogs and 3% (1) of non–dog owners and *hlyD* in isolates from 2% (1) of dog owners.

Agreement between virulence factors—Very good agreement existed between hemolytic phenotype and the *hlyD* virulence gene in fecal *E coli* isolates (κ = 0.884; $P < 0.001$). Eighteen percent (13/72) of isolates that possessed *hlyD* did not have a hemolytic phenotype.

In dogs, there was perfect agreement (κ = 1.000; $P < 0.001$) between detection of the *cnf* gene and detection of the *hlyD* gene in fecal *E coli* isolates. There was also very good agreement (κ = 0.886; $P < 0.001$) between detection of the *cnf* gene and detection of the *papGIII* gene and between detection of the *hlyD* gene and de-

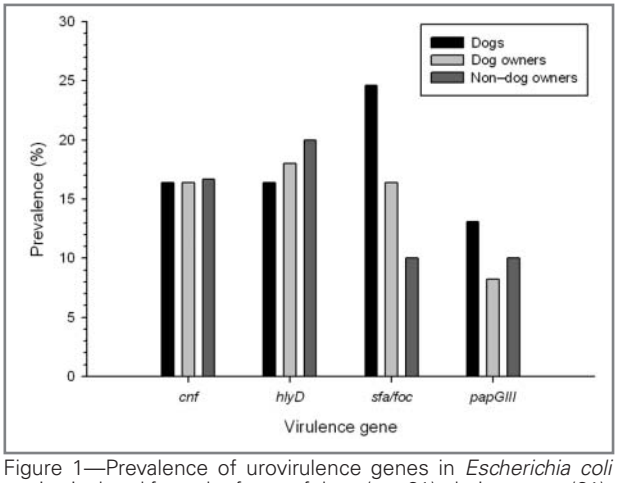


Figure 1—Prevalence of urovirulence genes in *Escherichia coli* strains isolated from the feces of dogs (n = 61), their owners (61), and non–dog owners (30).

tection of the *papGIII* gene in isolates. In dog owners, agreement between detection of the *cnf* gene and detection of the *sfa/foc* gene in fecal *E coli* isolates was perfect ($\kappa = 1.000$; $P < 0.001$), and agreement was very good between detection of the *cnf* gene and detection of the *hlyD* gene ($\kappa = 0.936$; $P < 0.001$) and between detection of the *hlyD* gene and detection of the *sfa/foc* gene ($\kappa = 0.936$; $P < 0.001$). In non–dog owners, agreement was very good ($\kappa = 0.870$; $P < 0.001$) between detection of the *cnf* gene and detection of the *hlyD* gene.

Comparison of virulence factors between groups—Fecal *E coli* isolates from dogs and their owners did not differ significantly with respect to the prevalence of any virulence gene. In 57% (35/61) of households, all isolates from dogs and their owners had negative PCR-assay results for all 4 virulence genes. In only 1 household did isolates from the dog and owner have ≥ 1 virulence gene in common. In that situation, all 3 fecal *E coli* isolates from the dog and owner possessed genes *cnf*, *hlyD*, and *sfa/foc*.

A significantly ($P = 0.002$) greater proportion of fecal *E coli* isolates from dogs (22% [40/183]) possessed the *sfa/foc* gene, compared with the proportion of isolates from non–dog owners (7% [6/90]), but there were no differences between these groups with respect to prevalence of the other 3 virulence genes. No differences were detected between dog owners and non–dog owners with respect to the prevalence of any virulence gene in fecal *E coli*.

Comparison of virulence factors with participant characteristics—Proportions of canine fecal *E coli* isolates in which virulence genes were detected did not differ between sexes. However, in humans, fecal *E coli* isolates from males (dog owners and non–dog owners combined) were significantly ($P = 0.010$) more likely to possess the *papGIII* gene than were isolates from females. Detection of virulence genes in *E coli* from dogs and dog owners was not associated with living in a single- or multiple-pet household, nor was it associated with owner's affiliation with the veterinary teaching hospital or a human hospital.

Prevalence of virulence factors in relation to UTI history—Fifty-two percent (37/71) of women and no men reported having a UTI during their lifetime. The difference in proportions was significant ($P < 0.001$). Of the women that had a history of UTI, 24% (9/37) reported 1 UTI during their lifetime, whereas 76% reported multiple UTIs. Eighteen percent (6/33) of female dogs and 7% (2/28) of male dogs had a history of UTI during their lifetime, but the difference in proportions was not significant ($P = 0.203$). Half (4/8) of dogs with a history of UTI had multiple UTIs.

In female human participants (dog owners and non–dog owners combined), a history of UTI was associated with presence of the *hlyD* ($P = 0.048$) or *papGIII* ($P = 0.026$) gene in their fecal *E coli* isolates; however, these associations were not significant when the Bonferroni correction was applied. In dogs, no association was detected between history of UTI and presence of any virulence gene in fecal *E coli*. History of multiple UTIs was associated with an increased likelihood of detecting the *papGIII* gene in fecal *E coli* isolates from women ($P = 0.008$) but not in isolates from dogs.

In female dog owners, a history of UTI was significantly associated with detection of each virulence gene in *E coli* isolates from their dog's feces (*cnf*, $P < 0.001$; *hlyD*, $P < 0.001$; *sfa/foc*, $P = 0.007$; and *papGIII*, $P < 0.001$). Female dog owners with history of UTI were more likely to have dogs with *E coli* isolates possessing virulence genes than were owners without history of UTI. No association was detected between a history of UTI in dogs and detection of virulence genes in their owner's fecal *E coli*.

Discussion

In the study reported here, 22% of canine and human participants had fecal *E coli* with 1 or more genes that encode uropathogenic virulence factors, whereas most participants (78%) had fecal *E coli* with negative PCR-assay results for all 4 virulence genes tested. The low prevalence of virulence genes in fecal *E coli* was not unexpected because these 4 virulence genes were chosen for their association with urovirulence but were tested in fecal isolates from healthy participants. In another study,[15] the prevalence of 1 or more urovirulence genes, including *cnf*, *hly*, *sfa/foc*, and *pap*, was only 35% in *E coli* from the fecal flora of healthy adult humans, compared with almost 70% in uropathogenic strains of *E coli* recovered from adult humans with a UTI, supporting the association of these particular genes with urovirulence.[15]

The prevalence of the *cnf* gene among the fecal *E coli* isolates in our study was comparable to results of other studies[5,16] of fecal isolates from healthy dogs (15% vs 12% to 30%, respectively), but slightly higher than the reported prevalence in fecal isolates from healthy humans (14% in dog-owner isolates vs 0.9% to 10% in other studies[16,17]). The prevalence of hemolysin gene *hlyD* in our study was comparable to that of other studies involving feces of healthy dogs[5,16] (15% vs 6% to 31%, respectively) and humans (16% in dog-owner isolates vs 2.7% to 20% in other studies[17,18]). The s-fimbrial adhesin gene was more prevalent in fecal *E coli* isolates from dogs (22%) than in isolates from non–dog owners (7%), as was found in another study[16] involving healthy humans and dogs. The prevalence of the *papGIII* gene in fecal *E coli* isolates in this study was comparable with the prevalence in other studies[5,9,18,19] (13% vs 6% to 30% in fecal isolates from healthy dogs and 9% vs 4% to 8.2% in fecal isolates from healthy humans). On the other hand, the prevalence of virulence genes in *E coli* isolated from the urine of humans or dogs with a UTI is much higher.[5,18]

The combination of 4 virulence factors, *cnf1, hly, sfa,* and *pap*, was the most common pattern identified in a study[20] of *E coli* in the urine of dogs and cats with a UTI. In another study,[21] 70% of *E coli* isolated from the urine of dogs with a persistent UTI possessed 1 or more virulence genes. Present in 28% of isolates, *papGIII* was the least common gene but was always detected in combination with genes *cnf*, *hly*, and *sfa/foc*. The commonness of 4 virulence genes being detected together in our and other studies suggested genetic linkage of all 4 on a pathogenicity island. Such pathogenicity islands have

been identified linking genes *cnf* and *hlyD* and genes *cnf*, *hlyD*, and *papGIII*.[22,23]

A possible explanation for the absence of a hemolytic phenotype despite presence of the *hlyD* gene in some fecal *E coli* isolates in the present study is a defect in the hemolysin operon or a defect in the transcriptional activator *rfaH*.[24] Results of our study were consistent with the reported linkage of genes *hlyD* and *cnf*.[16,22,25] In our study, the *cnf* gene was detected in a large proportion (92%) of hemolytic isolates but was not detected in any nonhemolytic isolates; only the isolates from 2 participants had negative PCR-assay results for the *cnf* gene but positive results for the *hlyD* gene. Perfect agreement between detection of genes *cnf* and *hly* in the same isolate could be explained by linkage of the genes on a chromosomal pathogenicity island that governs the synthesis of both toxins, and these genes have been linked in *E coli* on pathogenicity island II_{J96}.[16,22,25] The strong agreement between detection of genes *cnf* and *sfa/foc* in the same isolate in the present study also suggests direct genetic linkage, whether on chromosomes or mobile genetic elements such as pathogenicity islands or plasmids. Virulence genes *cnf* and *sfa/foc* are commonly detected in combination with other virulence genes and are included in pathogenicity islands, although direct linkage to each other has not been established.[15,20,25]

Virulence gene results for dogs and owners within the same households did not differ significantly. Whereas low overall prevalence of the virulence genes and low power in the study may have contributed to the lack of significant findings, this lack cannot be explained by cross-species transmission of mobile genetic elements because there was only 1 household in which fecal *E coli* isolates from the dog and its owner had ≥ 1 virulence gene in common. There may be clinical importance to the finding that 41% of households had a dog or owner (but not both) with fecal *E coli* in which at least 1 virulence gene was detected. This suggests that even if fecal *E coli* carried mobile genetic elements or pathogenicity islands containing virulence genes, these genes were not transmitted routinely between fecal *E coli* in dogs and their owners in the present study.

In the women in the present study, a history of UTI was associated with presence of genes *hlyD* and *papGIII* in fecal *E coli* and history of multiple UTIs was associated with presence of the *papGIII* gene in fecal *E coli*. However, it must be considered that the strains of *E coli* present in feces at the time of collections did not necessarily represent the strains of *E coli* that might have been present at the time of the UTI. Of the 4 virulence genes analyzed in our study, *hlyD* and *papGIII* are most specific to the urogenital tract, whereas *cnf* is often present in intestinal pathogenic *E coli* and *sfa/foc* is also commonly associated with meningitis and bacteremia.[26,27]

The association identified between detection of each virulence gene in fecal *E coli* from dogs and their female owner's history of UTI has not been reported elsewhere. One possible interpretation of these results is that dogs may harbor *E coli* with urovirulence factors that may be transmitted, directly or indirectly, to their human housemates, thus increasing the owner's risk of acquiring a UTI from exposure to uropathogenic *E coli*. Two longitudinal studies revealed within-household sharing of *E coli* strains between women with a UTI and other family members or with their dog or cat.[28,29] Detection of genes *pap*, *hly*, and *cnf* was associated with persistence of these strains in feces of family members and pets after the women's UTIs had been treated and cleared, suggesting that other housemates and pets could be reservoirs for future reinfection.[28,29] In our study, although a significant association was detected between female dog owners having a history of UTI and their dogs having fecal *E coli* with virulence factors, it must be stressed that the owners did not have an active UTI at the time their dog's feces were collected. To better investigate the possible association between uropathogenic *E coli* in canine feces and UTIs in dog owners, fecal specimens collected from dogs and their owners at the time of the UTI should be analyzed for the presence of uropathogenic strains of *E coli*.

a. BBL CultureSwab Plus, Becton Dickinson & Co, Sparks, Md.
b. MUG Fluorescence Crystals, Hach Chemical Co, Loveland, Colo.
c. EC Medium with MUG, Becton Dickinson & Co, Sparks, Md.
d. API 20E test kits, bioMérieux Inc, Durham, NC.
e. Fisher Scientific, Fair Lawn, NJ.
f. NanoDrop ND-1000, NanoDrop Technologies Inc, Wilmington, Del.
g. Provided by JR Johnson, University of Minnesota, Minneapolis, Minn.
h. Applied Biosystems, Foster City, Calif.
i. Amplitaq Gold polymerase, Applied Biosystems, Foster City, Calif.
j. Ninety-six–well PCR plates, BioRad Laboratories, Richmond, Calif.
k. Microseal film, BioRad Laboratories, Richmond, Calif.
l. iCycler, BioRad Laboratories, Richmond, Calif.
m. Blue/orange loading dye, Promega, Madison, Wis.
n. 100-bp DNA ladder, Promega, Madison, Wis.
o. FPQuest Software, version 4.5, BioRad, Richmond, Calif.
p. SPSS, version 15.0, SPSS Inc, Chicago, Ill.

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