Phylogenetic and Pathotypic Similarities between Escherichia coli Isolates from Urinary Tract Infections in Dogs and Extraintestinal Infections in Humans

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Seventeen Escherichia coli isolates from dogs with urinary tract infection (UTI) were characterized with respect to phylogenetic background and virulence genotype and were compared with the E. coli reference (ECOR) collection and with human clinical isolates with similar serotypes from patients with diverse extraintestinal infections. Most of the canine urine isolates were from (virulence-associated) E. coli phylogenetic groups B2 or D, expressed papG allele III, and exhibited numerous other putative virulence genes that are characteristic of human extraintestinal pathogenic E. coli (ExPEC). Close phylogenetic and pathotypic correspondence were documented within 5 clonal groups among individual canine and human isolates, including archetypal human ExPEC strains CFT073 (O6:K2:H1), 536 (O6:K15:H31), and J96 (O4:K−:H5). These findings suggest that canine UTI isolates, rather than being dog-specific pathogens, as previously suspected, may pose an infectious threat to humans. Commonality between canine and human ExPEC has potentially important implications for disease prevention, antibiotic resistance avoidance, and studies of pathogenesis.

The virulent strains of Escherichia coli that cause genitourinary infections in dogs and cats are significant because of both their health consequences for these animal hosts [1] and the possibility that they may be pathogenic for humans and may even be acquired by humans from domestic pets [1–5]. A strain collection of historical significance with respect to contemporary understandings of the relationships between human and canine isolates of extraintestinal pathogenic E. coli (ExPEC) [6] comprises the 17 E. coli isolates from dogs with urinary tract infection (UTI) that were first described by Garcia et al. [7] in 1988. These canine UTI isolates, although exhibiting O:K:H serotypes associated with human ExPEC and expressing fimbrial (F) antigens consistent with (human) pyelonephritis-associated P fimbriae, exhibited diverse “atypical” agglutination phenotypes that differed from those exhibited by P fimbriated human ExPEC controls [7] and adhered to canine, not human, uroepithelial cells [8]. This suggested that, despite their many similarities, human and canine ExPEC might differ in a host species–specific manner with respect to key virulence factors (VFs) [1, 7–10].

From 1 of these canine UTI isolates, strain 1442, Marklund et al. [11] later cloned a pap (P fimbrial) operon that contained a version of the papG adhesin gene (termed prsG, papG allele III, or pap-2) that was distinct from that found in most of the pap-positive human isolates that had been studied to date. This new pap (or prs) operon from strain 1442 conferred agglutination and adherence phenotypes consistent with those exhibited by other canine E. coli isolates but not by pap-positive human-source E. coli controls [9–11]. These and similar observations led to the concept, which has prevailed over the past decade, that human and canine ExPEC represent distinct populations, each with its own host species–specific variant of the P fimbrial adhesin molecule PapG [1, 8–10, 12]. PapG variant III, however, is now known to be the predominant PapG variant among E. coli isolates from humans with cystitis [13, 14] and to occur also among isolates from patients with pyelonephritis [15], prostatitis [16], neonatal meningitis (J. R. J., unpublished data), and diverse-source E. coli bacteremia [17, 18]. Evidence recently was presented that canine- and human-derived versions of papG allele III are essentially indistinguishable with respect to peptide sequence and agglutination phenotypes [4]. Clonal overlap was demonstrated among human and canine ExPEC isolates of serogroups O4 and O6 that contained papG allele III [2, 4]. The highly similar human and
canine strains also contained the F48 allele of \textit{papA} (the P fimbrial structural subunit gene) and multiple other putative VF genes associated with human ExPEC, including \textit{sfa}/\textit{foc} (SI-F1C fimbriae), \textit{bfa} (hemolysin), \textit{cnf1} (cytotoxic necrotizing factor), \textit{fyuA} (yersiniabactin), and \textit{kpsM}T-II (group II capsules) [2, 4].

These observations suggested a need to use contemporary molecular and phenotypic methods to reanalyze the classic collection of canine UTI isolates from Garcia et al. [7]. In the present study, we sought to determine the molecular basis for these strains’ diverse mannose-resistant hemagglutination (MRHA) phenotypes. We also sought to characterize the strains with respect to a broad range of VFs associated with human ExPEC, to define their phylogenetic background in comparison with control strains from the \textit{E. coli} reference (ECOR) collection [19], and to identify possible matches between the canine isolates and clinical \textit{E. coli} isolates from humans.

\textbf{Methods.} The canine strains studied were the 17 urine isolates from dogs with UTI, as analyzed by Garcia et al. [7]. Published data for these strains included O:K:H serotypes (as determined by the International \textit{E. coli} Centre, Statens Serum Institut); P fimbrial F antigens (as determined by crossed immunoelectrophoresis using F7-1, F7-2, and F8-F16 antisera and by whole-bacteria ELISA using monoclonal antibodies specific for F7-1, F7-2, F8, F9, F12, F13, or F16 fimbriae); and MRHA phenotypes with dog, human, dog, rabbit, guinea pig, sheep, pig, horse, and bovine erythrocytes [7]. The 5 serogroup O6 members of the collection were assessed previously for esterase allozymes and outer membrane protein profiles [20].

The canine isolates were compared with diverse clinical isolates from humans with pyelonephritis, cystitis, pyelonephritis, bacteremia, diarrhea, and neonatal meningitis, which were selected from the investigators’ collections on the basis of their known serological and/or pathotypic similarities to certain of the canine isolates. They included neonatal meningitis isolates 971527 and 931548, from the Netherlands (provided by Lodewijk Spanjaard, Department of Medical Microbiology, Academic Medical Center, The Netherlands Reference Laboratory for Bacterial Meningitis, Amsterdam); acute cystitis isolates 272, 1194, and F8, from adult women in Seattle (provided by Ann Stapleton, University of Washington) [14]; pyelonephritis isolate CFT073, from Baltimore (provided by H. L. T. Mobley, University of Maryland) [21]; pyelonephritis isolate 536, from Würzburg, Germany (provided by Gabrielle Blum-Oehler, Universität Würzburg) [22]; pyelonephritis isolate J96, from Seattle (provided by Barbara Minshew, formerly of the University of Washington) [23]; bacteremia isolates BOS002 and BOS068, from veterans in Boston (provided by Joel Maslow, Philadelphia Veterans Affairs Medical Center, Philadelphia) [24]; acute cystitis isolate CU141, from a woman in Minneapolis (present study; J.R.J.); UTI isolate H35, from Spain (provided by Jorge Blanco, Universidad Santiago, Lugo, Spain); diarrhea isolate 821-80 (provided by Nancy Stockbine, Centers for Disease Control and Prevention, Atlanta); and urosepsis isolates U5, U6, U7, V9, and PM9, from adults in Seattle [25, 26]. The phylogenetic controls included ECOR control strains that represented phylogenetic groups A (ECOR 4), B1 (ECOR 71), B2 (ECOR 52 and ECOR 62), D (ECOR 39), and the nonaligned strains (“non”; ECOR 31), as defined on the basis of neighbor-joining analysis of electrophoretic polymorphisms for 38 metabolic enzymes [27]. The ECOR strains were obtained from Howard Ochman (University of Arizona, Tucson) [19] and the American Type Culture Collection. Strains were stored at −70°C in 15% glycerol and broth until use.

\textbf{Phytophony by random amplified polymorphic DNA (RAPD) analysis.} RAPD patterns were generated for each strain by using (separately) arbitrary decamer oligonucleotide primers 1247, 1281, and 1283 [28], with polymerase chain reaction (PCR) conditions, as described elsewhere [29], except that commercial PCR beads (“Ready to Go”; Pharmacia) were used [4]. Gel images were captured and were analyzed by using a digital system (Gel Doc and Molecular Analyst, BioRad), as described elsewhere [4, 30]. Similarity matrices were constructed on the basis of Pearson’s correlation coefficient analysis of pairwise comparisons of single-primer RAPD patterns and of composite “virtual” RAPD patterns, which were derived by digitally combining the individual single-primer RAPD patterns for each strain in a head-to-toe fashion [4, 30]. Each canine isolate was presumptively assigned to the phylogenetic group of the ECOR control strain to which the canine isolate was closest in the similarity matrices. Because of discrepancies between the various RAPD primers with respect to the specific phylogenetic group assignment of several strains (not shown), phylogenetic designations were limited to 2 broad groups. The first (group 1) corresponded with ECOR phylogenetic groups B2 and D, and the second (group 2) corresponded with ECOR groups A, B1, and “non” [27].

\textbf{VF genotyping.} Strains were tested for 30 putative VF genes of ExPEC by use of a multiplex PCR assay, as described elsewhere [4, 26]. Probe hybridization also was used for \textit{kpsM}T-II, as described elsewhere, to detect the K2 \textit{kpsM}T variant, which does not react with the \textit{kpsM}T II primers used [26]. Primers for open reading frame R4 (\textit{malX}) were used as a marker for a sequenced pathogenicity-associated island (PAI) from archetypal ExPEC strain CFT073 (O6:K2:H1) [31, 32], as described elsewhere [2, 4, 26, 33]. Strains were tested for the 12 known alleles of \textit{papA} by use of an F type–specific multiplex PCR assay, as described elsewhere [4, 34]. Boiled lysates were used as template DNA. All assays were done at least in duplicate, using independently prepared lysates, with any discrepancies investigated further as needed.

\textbf{Cluster analysis of VF profiles.} The extended VF genotypes of the 17 canine isolates, including \textit{papA} and \textit{papG} alleles, were subjected to cluster analysis, according to the unweighted pair group method with averaging (UPGMA) [35], by use of the numerical taxonomy and multivariate analysis system (NTSYS; Exeter Software).

\textbf{MRHA phenotypes.} MRHA was assessed by using dense suspensions of plate-grown bacteria in microscope slide assays at 4°C, as described elsewhere [36, 37]. Erythrocytes used were human (AP, J.R.J.), sheep, dog, and pig. MRHA with each erythrocyte type was assessed in the presence of 1 added volume of PBS (pH 7.4; negative control inhibitor) or of supernatant of centrifuged whole pigeon egg white, a digalactoside-containing inhibitor of P fimbrial binding [38]. Assays were interpreted without reference to the ad-

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Figure 1. Characteristics of 17 canine urinary tract infection isolates of *Escherichia coli*. Serotypes are as published elsewhere [7], although strain 1520 was described by Cherifi et al. [20] as K33 rather than K13 (indicated by an asterisk [*]). All strains were negative for *papG* alleles I and II, *afa/draBC*, *bmaE*, *gafD*, *nfaE*, *iha*, and *kpsMT-III* and gave concordant results for *kpsMT-II* by blot, compared with polymerase chain reaction analysis. Mannose-resistant hemagglutination (MRHA) for human (Hu), sheep (Sh), dog (Do), and pig (Pi) erythrocytes is indicated. Plus signs (+) indicate the presence of a trait, and minus signs (−) indicate the absence of a trait. Strains are listed in the same sequence as they appeared in the unweighted pair group method with averaging dendrogram based on virulence factor profiles (not shown). The horizontal rule separates phylogenetic “group 1” from “group 2,” as defined by random amplified polymorphic DNA (RAPD) analysis. Non-P, non-P-pattern; P, P-pattern.

### Results

**Phylogenetic relationships among the canine UTI isolates.** According to RAPD analysis, the 17 canine UTI isolates segregated into 2 broad phylogenetic groups (figure 1). Twelve (71%) of the isolates corresponded most closely with controls from ECOR groups B2 and D, which are associated with extraintestinal virulence in humans [39, 40]. The remaining 5 isolates (29%) corresponded best with controls from ECOR groups A, B1, or “non,” which are associated chiefly with commensal status [19, 27, 40]. A greater proportion of the canine UTI isolates (figure 1) than of the ECOR strains [19, 27] represented virulence-associated phylogenetic groups B2 and D (71% vs. 38%; *P* = .016).

O-K:H serotypes for the 17 canine isolates that were determined elsewhere [7] corresponded with this inferred phylogenetic distribution. The virulence-associated O2, O4, O6, and O75 antigens [41] accounted for 3 of the 5 phylogenetic isolates from ECOR groups B2 and D, which are associated with extraintestinal virulence in humans [39, 40]. The remaining 5 isolates (29%) corresponded best with controls from ECOR groups A, B1, or “non,” which are associated chiefly with commensal status [19, 27, 40]. A greater proportion of the canine UTI isolates (figure 1) than of the ECOR strains [19, 27] represented virulence-associated phylogenetic groups B2 and D (71% vs. 38%; *P* = .016).

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### Prevalence of VF genes.

Of the 30 human ExPEC-associated VF genes analyzed, all but 8 (*papG* alleles I and II, *afa/draBC*, *bmaE*, *gafD*, *nfaE*, *iha*, and *kpsMT-III*) were detected in ≥1 of the 17 canine UTI isolates. *pap* and *sfa/foc* were both highly prevalent and were the only mannose-resistant adhesin genes encountered. Of the several *papA* alleles that were de-
ected, F12 was the most prevalent (n = 5), followed by F10 (n = 3). papG allele III was the only papG allele detected (P < .01 vs. alleles I or II, McNemar’s test).

**Comparison with virulence genotypes of ECOR strains.** Compared with the 72 human and animal isolates of the ECOR collection, the 17 canine UTI isolates exhibited a higher prevalence of multiple VF genes, including papAH, papC, papEF, papG, papG allele III, sfa/foc, hlyA, cnf1, cdtB, fyuA, iroN, and malX (table 1). In contrast, they exhibited a lower prevalence of iha (table 1).

**Phylogenetic distribution of VF genes.** Among the canine UTI isolates, the VF genes exhibited a striking phylogenetic distribution, analogous to that previously demonstrated among human urosepsis isolates [26]. VFs that were strongly associated with phylogenetic group 1 included pap elements, sfa/foc, hlyA, cnf1, kpsMT-II, and malX (P < .003 for all comparisons; figure 1). Several other VF genes, including sfaS, focG, cdtB, rfc, and ibeA, were confined to group 1 (figure 1; table 1). In contrast, 3 VFs were more prevalent and/or occurred only in group 2, including cvaC (P = .015 for association with group 2), traT (P = .053), and iutA (P = .07).

According to UPGMA cluster analysis of VF profiles (not shown), which was done independently of genomic background, the 17 canine isolates segregated into 2 distantly related VF profile clusters. One of the clusters comprised the 10 pap-positive strains from phylogenetic group 1. The other cluster comprised the 2 pap-negative strains from phylogenetic group 1 plus all 5 group 2 strains.

**Correlations between VFs.** Significant correlations between individual VFs were noted among the canine isolates in patterns reminiscent of those recently described among human urosepsis isolates [26] (table 2). Two major associative sets of VFs were evident, with the members of each set exhibiting positive associations with other VFs within the same set and negative associations with VFs from the other set. The first set comprised the group 1–associated VFs pap, sfa/foc, hly, cnf1, iroN, kpsMT-II, and malX (table 2). cdtB approached statistical membership in this group (P = .056 vs. papG; P = .09 vs. hlyA and cnf1). The second set comprised the group 2–associated VFs iutA, cvaC, and traT (table 2). In addition, ibeA and the K1 kpsMT variant were highly correlated with one another but not with the VFs from either of the 2 major sets (table 2).

**MRHA phenotypes.** The newly determined MRHA patterns of the 17 canine isolates corresponded almost precisely with papG allele genotypes (figure 1). With 1 exception, all strains (and only those strains) that contained papG allele III exhibited P-pattern MRHA of human, sheep, pig, and dog erythrocytes. The exception, strain 1612, contained sfa/foc and sfaS, in addition to papG allele III, and exhibited non–P-pattern MRHA of human and sheep erythrocytes but P-pattern MRHA of dog and pig erythrocytes. The P-pattern MRHA phenotypes exhibited by the papG-positive canine isolates were indistinguishable from those of the human-source papG allele

<table>
<thead>
<tr>
<th>VF</th>
<th>Canine UTI isolates (n = 17)</th>
<th>ECOR strains (n = 72)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>papAH</td>
<td>12 (71)</td>
<td>22 (31)</td>
<td>.004</td>
</tr>
<tr>
<td>F12 papG allele</td>
<td>5 (29)</td>
<td>3 (4)</td>
<td>.006</td>
</tr>
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<td>papG</td>
<td>10 (59)</td>
<td>19 (26)</td>
<td>.02</td>
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<td>Allele III</td>
<td>10 (59)</td>
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<td>sfa/foc</td>
<td>12 (71)</td>
<td>11 (15)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>sfaS</td>
<td>2 (12)</td>
<td>1 (1)</td>
<td></td>
</tr>
<tr>
<td>focG</td>
<td>4 (24)</td>
<td>9 (13)</td>
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<tr>
<td>iha</td>
<td>0</td>
<td>23 (32)</td>
<td>.005</td>
</tr>
<tr>
<td>hlyA</td>
<td>11 (65)</td>
<td>9 (13)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>cnf1</td>
<td>11 (65)</td>
<td>8 (11)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>cdtB</td>
<td>4 (24)</td>
<td>2 (3)</td>
<td>.01</td>
</tr>
<tr>
<td>fyuA</td>
<td>16 (94)</td>
<td>46 (64)</td>
<td>.02</td>
</tr>
<tr>
<td>iutA</td>
<td>2 (12)</td>
<td>21 (29)</td>
<td></td>
</tr>
<tr>
<td>iroN</td>
<td>15 (88)</td>
<td>11 (22)</td>
<td>&lt;.001</td>
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<tr>
<td>kpsMT II</td>
<td>10 (59)</td>
<td>28 (39)</td>
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<tr>
<td>K1</td>
<td>2 (12)</td>
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<td>rfc</td>
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<td>cvaC</td>
<td>3 (18)</td>
<td>3 (4)</td>
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<tr>
<td>traT</td>
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</tr>
<tr>
<td>malX</td>
<td>13 (76)</td>
<td>21 (29)</td>
<td>&lt;.001</td>
</tr>
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</table>

**NOTE.** Data are no. (%) of isolates with associated VF. All canine isolates were negative for papG allele II, sfa/foc, hlyA, cnf1, kpsMT-II, and malX. All were positive for iha and sfa/S. All yielded concordant results for kpsMT III with probe hybridization, compared with polymerase chain reaction analysis. Data for ECOR strains are from [30].

* a For canine isolates, results for papG allele II, sfa/foc, hlyA, cnf1, kpsMT-II, and malX were indistinguishable from those of the anticipated canine isolates as predicted, with no greater detectable difference between the canine and human isolates within each cluster than could be accounted for by technical factors (figure 2). VF profiles were more similar overall among isolates within a given cluster, irrespective of host species, than they were among isolates from different clusters, even if from the same host species (figure 3).

Cluster analysis of the VF data from figure 3 according to

II and III controls but distinct from those of the papG allele I and sfa controls (data not shown).

**Comparison with human ExPEC isolates.** On the basis of serotypes, VF profiles, and phylogenetic data regarding the 5 canine isolates from serogroup O6 determined elsewhere [20], for 13 of the canine isolates, 1 putative matches were identified within the investigators’ collections of human clinical isolates. In all, 19 human isolates from patients with urosepsis, diverse-source bacteremia, neonatal meningitis, pylonephritis, diarrhea, and cystitis were compared directly with the 13 canine isolates by RAPD analysis.

In the resulting RAPD dendrogram, the canine isolates and 17 of the 19 human isolates segregated into 5 clusters (i.e., putative clonal groups), each of which included representatives from both host species (figure 2). Human isolates clustered with the anticipated canine isolates as predicted, with no greater detectable difference between the canine and human isolates within each cluster than could be accounted for by technical factors (figure 2). VF profiles were more similar overall among isolates within a given cluster, irrespective of host species, than they were among isolates from different clusters, even if from the same host species (figure 3).
Discussion

In the present study, we used contemporary molecular and phenotypic methods to characterize a classic set of canine UTI isolates [7, 8] and to compare these strains with human-source ExPEC. We found numerous phylogenetic and pathotypic similarities between the canine isolates and clinical isolates from humans, both in the aggregate and at the level of individual strains. These findings support the hypothesis that canine- and human-source ExPEC represent overlapping populations, with members of certain clones or clonal groups capable of causing infections in both host species.

The observed predominance of papG allele III among the canine isolates in the present study is consistent with the previous demonstration of papG allele III in strain 1442 [11]. It corresponds with the early observation by Marklund et al. [11] that pap-positive canine fecal E. coli isolates contained only papG allele III and with the recent demonstration of papG allele III as the most prevalent papG allele within a small set of canine urine and fecal isolates [4]. That Marklund et al. [11] found only papG allele II among their human-source controls suggested to those investigators that papG allele III is specific to dogs and that papG allele II is specific to humans [9–11]. It is now known, however, that papG allele II is the most prevalent papG allele among E. coli isolates from humans with cystitis [13, 14] and, in some reports, is appreciably prevalent also among isolates from patients with prostatitis [16], bacteremia [17, 18], and pyelonephritis [15, 42]. Thus, the notion that pap-positive canine strains are irrelevant for human disease because of their supposed “dog-specific” papG variant is not tenable.

Previously, the canine isolates examined in the present study were found to exhibit MRHA phenotypes that were diverse and dissimilar to those of human-source comparison strains [7]. In contrast, we found that the pap-positive canine isolates exhibited a largely uniform P-pattern MRHA phenotype that was indistinguishable from that of the human-source controls for papG alleles II and III. The only evidence of phenotypic diversity among the canine isolates involved a single sfaS-positive strain (strain 1612) that, like the sfa control strain, exhibited pigeon egg white-resistant (i.e., non-P) MRHA of human and sheep erythrocytes. These discrepancies between historical and newly determined MRHA phenotypes may relate to the use in the different studies of different methods, observers, and erythrocyte donors [7]. The importance of the erythrocyte donor was illustrated by the conflicting MRHA results previously obtained for 2 of the 17 canine isolates with erythrocytes from a Danish dog, compared with a Dutch dog [7]. Of note, we used an inhibitor of P fimbriae to clarify the specificity of MRHA, whereas digalactoside-coated beads, which are no longer available and are insensitive for detecting expression of papG allele III [4, 43], were used by Garcia et al. [7] to detect digalactoside-specific (P fimbrial) binding.

Our findings suggest considerable commonality between human and canine ExPEC. This conclusion is consistent with certain previous reports [4, 5, 20, 44] and conflicts with the notion that has prevailed until recently that human and animal strains of E. coli represent distinct populations [1, 12, 45]. Specifically, within 5 clonal groups, we found evidence of close similarity between canine isolates and human isolates from patients with neonatal meningitis, bacteremia, diarrhea, and cystitis. The 5 F12-positive isolates from our phylogenetic group I, which included the canine prs source strain 1442, equate with clonal group I, as defined by Cherifi et al. [20], which comprised isolates from both humans and diverse animal species. As we
Dendrogram from random amplified polymorphic DNA (RAPD) analysis of selected canine and human *Escherichia coli* isolates. Composite RAPD patterns from primers 1247, 1254, and 1281 were used to construct the dendrogram according to the unweighted pair group method with averaging. The 5 major clusters (putative clonal groups), each of which is composed of both canine (D) and human (H) isolates, are demarcated with brackets and are labeled according to the consensus O antigen(s) of the constituent strains. The 100-bp ladder lanes (MW) form an outgroup cluster that reflects the variability inherent in gel electrophoresis and image analysis. The gel tracks as shown are digital reconstructions and hence underestimate the resolution of the actual gel images. Two human isolates did not correspond closely with the canine isolates and therefore are not shown.

anticipated on the basis of its serotype, archetypal human ExPEC strain CFT073 [21] also is a member of this group, although it exhibits a distinctive virulence profile (e.g., presence of *papG* allele II instead of III and absence of *cnf1*), which differs from that of the non-K2 human and canine isolates from this clonal group [20], but is characteristic of many human-source O6:K2:H1 strains [26].

A clonally distinct O6 isolate from our phylogenetic group 1, strain 1655 (O6:K14:H31:F48), equates with clonal group 2 of Cherifi et al. [20], which likewise included both human and animal isolates. As we anticipated, the cluster that included strain 1655 also included archetypal human ExPEC strain 536 [46] and urosepsis isolate U7 [26], the source strain for the F48 *papA* allele [34] and the first human isolate shown by RAPD analysis and pulsed field gel electrophoresis to closely match a canine UTI isolate [4]. These 2 serogroup O6 clonal groups of Cherifi et al. [20] correspond with lineages 42 and 47, respectively, within (phylogenetic group B2-equivalent) cluster III of the collection of human bacteremia isolates reported by Maslow et al. [24] and represent the 2 largest clonal groups within that collection (J. R. J., unpublished data). They also correspond with electrophoretic types 1 and 21, respectively, in the study by Whittam et al. [44] of clonal overlap between human and animal extraintestinal *E. coli* isolates [2].

The 4 O4:K-:H5 clonal group members (figures 2 and 3) exhibited traits suggestive of the recently described “J96-like”
### Figure 3. Characteristics of phylogenetically related *Escherichia coli* isolates from dogs (bold; n = 13) and humans (n = 17). Isolates are listed in the same sequence as in the random amplified polymorphic DNA dendrogram (figure 2), with horizontal rules separating the 6 major clonal groups. aClusters O6 (a) and O6 (b) represent 2 related, yet genetically distinct, clonal groups of O6 isolates. bHost codes: D, dog; H, human. Source codes: B, bacteremia; C, cystitis; D, diarrhea; M, neonatal meningitis; P, pyelonephritis; U, urinary tract infection. All strains were negative for bmaE, gafD, nfaE, afa/draBC, and kpsMT III. cStrain U6 (O8:K27:H-) was positive for papAH by blot testing but not by polymerase chain reaction (PCR) analysis and was negative in the F PCR assay. dStrain CFT073 was positive for kpsMT II by blot testing only, which is consistent with the presence of the K2 kpsMT II variant.
clonal group that includes archetypal human ExPEC strains J96 and CP9 [23, 47]. Characteristics that would place these 4 strains with J96-like O4s, rather than with other O4s, include the presence of sfa / foc and cnf1 and the absence of iutA [23, 47]. Although most reported members of the J96-like clonal group have papG alleles I and III and the F13 papA allele (as is the case for both J96 and CP9), some members have papG allele III alone or with allele II and lack the F13 papA allele [23, 47]. The O4:H5 strains from the present study lacked the signature kpsMT III variant of the J96-like clonal group but, consistent with their capsule-minus status, also were negative for kpsMT II, which is typically present in non-J96-like O4s [23, 47]. RAPD analysis showed the O4:H5 strains from the present study to be indistinguishable from strain J96, which supports their possible membership in the J96-like clonal group.

Of note, 1 of the O4:H5 strains was isolated from a human with diarrhea. Whether some “ExPEC” strains are capable of causing intestinal, as well as extraintestinal, infections is an intriguing possibility that is suggested by findings pertaining to certain O4 and O6 E.coli isolates from dogs with diarrhea (J. R. J., A. L. S., and W. G., unpublished data).

These findings of close phylogenetic and pathotopic commonality between canine and human ExPEC have several implications. First, humans may acquire pathogenic E.coli from dogs, a process that may contribute to human disease [1, 48, 49]. There is ample precedent for fecal-oral transmission of other pathogens from pets to humans [50]. If this applies also to ExPEC, interruption of such zoonotic transmission may be a useful new method for preventing E.coli infections in humans. Transmission in the reverse direction also may occur, with adverse consequences for pets. Second, similar pathogenetic mechanisms are probably operative in dogs and in humans infected with such strains. Consequently, vaccines designed to protect one host group against these strains or their VF s [51–53] may have cross-protective efficacy in the other host group. Likewise, dogs may be well suited as hosts for experimental infection models in studies of UTI pathogenesis, because, unlike mice and monkeys, they appear to develop UTIs naturally due to strains that are essentially indistinguishable from certain human ExPEC. Finally, the potential for humans to become colonized and infected with canine-derived E.coli suggests that antibiotic usage practices in veterinary medicine could have significant implications for the selection of antimicrobial resistance among what eventually may become human pathogens [54]. Similarly, antibiotic usage practices in human medicine also could influence selection of resistance in potential animal pathogens.

The observed statistical associations between VF s are consistent with findings from other strain collections [14, 26]. Linkage on PAIs is a likely explanation for the observed associations between the phylogenetic group 1-associated VF s papG, hlyA, cnf1, iroN, kpsMT II, and malX. These genes are known to occur together in various combinations on the same PAIs in archetypal human ExPEC strains, such as J96, CP9, 536, RS218, AD110, and CFT073 [22, 31, 32, 46, 55–59]. By analogy to hly and cnf, the strong association of the F12 papA allele with cdtB, a diarrhea-associated toxin that recently has been documented also among human ExPEC isolates [26], suggests the possible colocalization of cdtB and an F12-containing pap operon on the same PAI in certain O6 strains. The observed association of the K1 kpsMT variant and ubeA mirrors that seen among human neonatal meningitis isolates [60, 61] and may reflect coselection for complementary VF s within certain lineages rather than a direct genetic linkage [57, 62, 63]. In contrast, linkage on colicin V plasmids is a more plausible explanation for the associations observed between the phylogenetic group 2 VF s cvaC, iutA, and traT [64, 65]. Among human urosepsis isolates, aerobactin plasmids are associated with compromised hosts, with cvaC, and with non-group B2 status [26, 66]. The group 2 canine isolates from the present study may represent the canine analogue of this phenomenon. This hypothesis is supported by the known presence of aerobactin plasmids in the 3 human urosepsis isolates from serogroups O8 and O9 (i.e., strains U6, V9, and PM9 [26, 66]) that, in the present study, were found to correspond with canine isolates 233, 3084, and 154 (figure 3).

In conclusion, in the present study, we characterized a well-known collection of canine UTI isolates with respect to phylogenetic background and virulence genotypes and phenotypes and compared these strains with selected human clinical isolates. We found that most of the canine isolates were from (virulence-associated) E.coli phylogenetic groups B2 and D, expressed papG allele III, and exhibited numerous other VF s characteristic of human ExPEC. Close phylogenetic and pathotypic correspondence was documented among certain canine and human isolates within 5 different clonal groups, several of which contained known archetypal human ExPEC strains. These findings suggest that canine UTI isolates, rather than being dog-specific pathogens, as previously suspected, may pose an infectious threat to humans. The considerable similarities between canine and human ExPEC have potentially important implications for disease prevention, antibiotic resistance avoidance, and studies of pathogenesis.

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ERRATUM

In 3 articles published in the Journal during 2001, the term “unpaired group method” should be replaced with “unweighted pair group method” as indicated in the following list.

