Evidence of Commonality between Canine and Human Extraintestinal Pathogenic Escherichia coli Strains That Express papG Allele III

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Although dogs have been proposed as carriers of extraintestinal pathogenic Escherichia coli (ExPEC) with infectious potential for humans, presumed host-species-differences between canine and human ExPEC strains have cast doubt on this hypothesis. The recent discovery that allele III of papG (the P fimbrial adhesin gene) predominates among human cystitis isolates and confers an adherence phenotype resembling that of canine ExPEC prompted the present reevaluation of the canine-human ExPEC connection. Sixteen paired papG-positive urine and rectal E. coli isolates from dogs with urinary tract infection were studied, papG (adhesin) and papD (piliin) allele type, agglutination phenotypes, virulence factor genotypes, and randomly amplified polymorphic DNA and pulsed-field gel electrophoresis fingerprints were analyzed and compared with those of human ExPEC controls. The 16 canine strains contained predominantly papG allele III. Agglutination phenotypes segregated strictly according to papG allele status and were homogeneous among strains with the same papG allele profile irrespective of their human versus canine origin. Canine and human PapG variant III peptide sequences were highly homologous, without host species-specific differences. The most prevalent canine papD allele was F48, a novel variant recently identified among human urosepsis isolates. In addition to papG, human ExPEC-associated virulence genes detected among the canine strains included sfa/focDE, sfa/S, fyuA, hlyA, cnf1,cdnB, kpsMT-II and -III, efc, traT, ompT, and a marker for a pathogenicity-associated island from archetypal human ExPEC strain CFT073. Molecular fingerprinting confirmed the fecal origin of all but one canine urine isolate and showed one pair of O6 canine urine and fecal isolates to be extremely similar to an O6 human urosepsis isolate with which they shared all other genotypic and phenotypic characteristics analyzed. These data demonstrate that canine ExPEC strains are similar to, and in some instances essentially indistinguishable from, human ExPEC strains, which implicates dogs and their feces as potential reservoirs of E. coli with infectious potential for humans.

Urinary tract infection (UTI) is a significant health problem for dogs as well as humans (25, 38, 39, 69, 75). Whether the extraintestinal pathogenic Escherichia coli (ExPEC) strains that cause UTIs and other extraintestinal infections in dogs are also capable of infecting humans is an important but unresolved question. Surveys of canine UTI isolates done approximately 10 years ago led to divergent conclusions regarding the relationship between canine and human ExPEC strains (5, 7, 41, 57, 71, 74). Westerlund et al. found canine ExPEC strains to exhibit a high prevalence of virulence-associated traits typical of human ExPEC strains, including digalactoside-binding P fimbriae (encoded by papG, for “pilus associated with pyelonephritis”), hemolysin (encoded by hly), and the O4 and O6 somatic antigens. Thus, these investigators proposed that canine ExPEC strains might pose an infectious threat to humans (71).

In contrast, subsequent investigators of canine ExPEC strains documented adherence patterns distinct from what at the time was expected of P-fimbriated human ExPEC strains. For example, unlike human pyelonephritis isolates, canine ExPEC isolates typically did not agglutinate digalactoside-coated latex beads (41, 57), ceramide trihexoside (CTH)-coated equine erythrocytes (74), or sometimes even human erythrocytes (particularly after neuraminidase treatment) (5, 41, 57), yet they did agglutinate canine and sheep erythrocytes, which the human ExPEC strain used as controls did not (5, 62).

Low et al. suggested that the atypical agglutination phenotype of most pap-positive canine ExPEC strains might be due to expression of P fimbriae containing a different adhesin molecule than that present in the P fimbriae of human ExPEC strains (41). Garcia et al. further proposed that this putative dog-associated P adhesin variant might be the novel adhesin described by Lund et al. as a second P fimbrial type in archetypal human pyelonephritis isolate J96 (5, 43). Stromberg et al. subsequently showed that this cloned prs (for “prs-related substance”) operon from strain J96 indeed conferred atypical adherence phenotypes similar to those exhibited by most pap-positive canine ExPEC strains but different from those of pyelonephritogenic human ExPEC. The prs-associated phenotype included agglutination of canine but not human erythrocytes, adherence to canine but not human epithelial cells, and binding to extended digalactoside-containing glycolipids, such as Forsman glycolipid (which is present in dogs but not humans), but not to CTH (which is present in humans but not dogs) (62). The further demonstration by Marklund et al. that archetypal canine ExPEC strain 1442 contains a prs-like operon seemed to establish prs as a distinctively dog-associated P fimbrial variant.
and to confirm canine ExPEC strains as pathogenetically irrelevant to humans (46).

Subsequent developments have necessitated a reappraisal of the importance for humans of the (prs-encoding) Prs adhesin variant, which is also known as Pap-2, Class III G adhesin, or PapG allele III; provided by Sheila Hull (16, 31); HU968-298 (S papG allele III; provided by Joel Maslow) (17, 48); P678-54/4787 (canine source), and 4787 (porcine source, F165 fimbriae) were included in the study, published PapG sequences for variant I from strain J96 (human source), 83972 (human source), 1442 (26). After column purification of papG amplicons, both strands were directly sequenced as previously described (26). When primer pGf (5′-TGTGTAATGGATATTTGT-3′) (32, 60, 64).

Materials and Methods

Strains. The 16 canine E. coli strains used were provided by the laboratory of P. A. Low et al. (52). PFGE fingerprints were generated for selected strains with each of five decamer oligonucleotide primers (16, 26, 27). PCR products were sequenced and automatically aligned using the application MEGA (34).

Genetic fingerprinting and cluster analysis of fingerprints. Since randomly amplified polymorphic DNA (RAPD) fingerprinting using multiple primers provides a reasonable proxy for multilocus enzyme electrophoresis (MLEE) for defining phylogenetic relationships among pathogenic E. coli (68), RAPD fingerprints were generated for selected strains with each of five decamer oligonucleotide primers (16, 26, 27). PCR products were resolved by size in ethidium bromide-stained agarose gels. OmpT, which encodes the outer membrane endopeptidase OmpT and is epidemiologically associated with extraintestinal virulence (4, 44, 61), was detected by dot-blot hybridization. Hybridization was done under stringent conditions as previously described (23), using a probe that was generated and digoxigenin-labeled by PCR using primers ompt-F (5′-ATCTAGCGGAAGAAGGAGGC-3′) and ompt-R (5′-CCCGG TCAATCCCTTTCATC-3′), which are based on the published ompT sequence (GenBank accession no. X06903). All genotyping assays were done at least in duplicate using template DNA isolated from dogs independently from separate colonies of each strain.

DNA sequencing and sequence alignment, and cluster analysis of sequences. papAH amplicons were generated by using forward primer PapAf (5′-ATGCCAGTACGTGCCCCCTC-3′), which has a recognition site within the 5′ end of papH, just downstream from papF (26). Full-length papG amplicons were generated by using forward flanking primer pGF (5′-CTGTAAATTACGAGAAGGTATTTCTG-3′) and reverse flanking primer pGR (5′-CTCGAGAATATCTGATTACG-3′; specific for alleles II and III) (26). After column purification of papAH and papG amplicons, both strands were directly sequenced as previously described (27). DNA sequences were translated into peptides and aligned using CLUSTALW (66). Dendrograms were inferred according to the neighborhood (NJ) method (54) using the application MEGA (34).

Agglutination assays. Digalactoside-coated latex beads were from Chembioford (Edmonton, Alberta, Canada; now defunct). Human P1, P2 and P3 phenol-soluble fimbriae were prepared by Jane Swanson, for ExPEC strains with each of five decamer oligonucleotide primers (16, 26, 27). DNA sequencing and sequence alignment, and cluster analysis of sequences. papAH amplicons were generated by using forward primer PapAf (5′-ATGCCAGTACGTGCCCCCTC-3′), which has a recognition site within the 5′ end of papH, just downstream from papF (26). Full-length papG amplicons were generated by using forward flanking primer pGF (5′-CTGTAAATTACGAGAAGGTATTTCTG-3′) and reverse flanking primer pGR (5′-CTCGAGAATATCTGATTACG-3′; specific for alleles II and III) (26). After column purification of papAH and papG amplicons, both strands were directly sequenced as previously described (27). DNA sequences were translated into peptides and aligned using CLUSTALW (66). Dendrograms were inferred according to the neighborhood (NJ) method (54) using the application MEGA (34).

For comparison with predicted PapG peptide sequences from the present study, published PapG sequences for variant I from strain J96 (human source), for variant II from strains AD110, DS17, GR12, and IA2, and for variant III from strains J96 (human source), 83972 (human source), 4787 (canine source), and 4787 (porcine source, F165 fimbriae) were included in the PapG dendrogram (6, 11, 33, 42, 45, 46, 62, 67). GenBank accession numbers for variant I from strain J96 (human source), 83972 (human source), 1442 (26). Full-length papG amplicons were generated by using forward flanking primer pGF (5′-CTGTAAATTACGAGAAGGTATTTCTG-3′) and reverse flanking primer pGR (5′-CTCGAGAATATCTGATTACG-3′; specific for alleles II and III) (26). After column purification of papAH and papG amplicons, both strands were directly sequenced as previously described (27). DNA sequences were translated into peptides and aligned using CLUSTALW (66). Dendrograms were inferred according to the neighborhood (NJ) method (54) using the application MEGA (34).

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Comparison of previous versus present analyses of canine ExPEC. In a previous study (27, 41), canine strains were tested for hemagglutination (HA) of human erythrocytes from a single donor (with and without neuraminidase digestion and in the presence and absence of inhibitors only of S fimbriae), and for agglutination of P beads. The present study included, in addition, erythrocytes representing multiple human blood phenotypes, control strains for the three papC alleles and for non-P-mannose-resistant (NPMR) adhesins, and specific inhibitors of both P and S fimbriae. Previously, pap status was analyzed as a dichotomous variable and hly was the only virulence factor studied other than pap (27, 41). In the present study, pap status was assessed by specific detection of four subregions of the pap operon, the three papG alleles, and the 12 papA alleles and by sequence analysis of papA and papG from selected strains. In addition, 23 non-pap virulence-associated genes were analyzed. Previously, phylogenetic relationships were inferred from O antigens, RFLP patterns for papA, hly, and IS5 and OMP profiles (27, 41). In the present study, cluster analysis of composite RAPD fingerprints was used to define the overall population structure and PFGE was used to assess close clonal relationships between strains.

Statistical methods. Comparisons of the prevalence of different virulence genes within the population were tested by using McNemar’s test, with a P value of <0.05 as the criterion for statistical significance.

Nucleotide sequence accession numbers. DNA sequences from the present study have been deposited in GenBank under accession numbers AF234626 (F48 papA control from strain U7), AF234627 (F10 papA control from strain V29), AF237472 through AF237475 (papG allele III from strains U7, C9, 1U, and 4R, respectively), and AF237478 through AF237482 (papA from strains 2U, 4U, 5U, 6R, and 8U, respectively).

RESULTS

RAPD fingerprinting. Analysis of the 16 paired urine and rectal E. coli by composite RAPD fingerprinting indicated that the corresponding rectal strain was most similar to the same host’s urine strain for seven of eight strain pairs (Fig. 2). These findings were concordant with previous typing results derived by other methods (41).

The RAPD dendrogram, which was used to delineate the underlying phylogenetic relationships between the strains (68), revealed two major clusters (Fig. 2). The first major cluster (designated clonal group A) comprised all five O4 strains. The second major cluster (clonal group C) comprised the four O6 strains plus the three Ont or O- strains that were paired with an O6 strain (Fig. 2; Table 1). Two pairs of urine and rectal strains (pairs 3U-3R and 2U-2R) were not included in either of these major clusters, but instead constituted independent clonal groups (B and D, respectively). Previously determined OMP profiles conformed to this phylogenetic distribution (Fig. 2; Table 1).

papG alleles. All 13 canine E. coli isolates which previously had exhibited the NS agglutination phenotype were found to contain papG allele III, as was the previously agglutination-negative urine strain (7U) which was paired with an NS phenotype rectal strain (7R) (Table 1). (The latter two strains also contained papG allele II in addition to allele III [Table 1].) In contrast, the two strains which previously had exhibited P-pattern agglutination (2U and 2R) were found to contain only papG allele II (Table 1). Thus, papG allele III was significantly more prevalent in the population than was papG allele II (P < 0.05, McNemar’s test), whether analyzed among all 16 isolates or only among the nine unique genotypes. Moreover, papG allele content corresponded with historical agglutination phenotypes.

Agglutination phenotypes. To determine whether agglutination phenotypes correspond most closely with host species or with papG allele content, agglutination phenotypes were reassessed for the 16 canine strains by using P latex beads, diverse erythrocyte types (with or without neuraminidase pretreatment), and various inhibitors of agglutination, in comparison with a panel of human-derived control strains containing papG alleles II or III, S fimbriae, or Dr family adhesins. As previously observed (41), only two of the canine strains (2U and 2R) agglutinated P beads; these were the two strains containing only papG allele II. Similar results were obtained with the control strains, of which only the papG allele II controls were P-bead positive (Table 2).

Irrespective of papG allele content, all of the canine strains (other than the previously HA-negative strain) and all of the control strains exhibited mannose-resistant HA (MRHA) of both A1P1 and OP1 human erythrocytes (Table 2). Pigeon egg white inhibited MRHA for all the HA-positive canine strains and for the papG allele II- and III-containing control strains but not for the Sfa and Dr control strains, evidence that MRHA as expressed by canine papG allele III-containing strains is digalactoside specific, i.e., is due to P fimbriae rather than to non-P MR adhesins. Fetuin inhibited MRHA for the Sfa control strain but for none of the canine or other control strains, evidence against S fimbriae as mediators of MRHA by the pap-positive canine and control strains.

In experiments done to further characterize the NS agglutination phenotype, for strains containing papG allele III (irrespective of their human versus canine origin), neuraminidase pretreatment of the erythrocytes yielded reduced MRHA intensity with OP1 but not A1P1 human erythrocytes (Table 2). In
contrast, for the Sfa control strain, neuraminidase pretreatment reduced MRHA intensity with both OP1 and A1P1 erythrocytes, whereas for the strains containing \textit{papG} allele II only and for the Dr control strain, neuraminidase pretreatment had no effect on MRHA intensity with either erythrocyte type (Table 2). These findings confirmed an association of the NS agglutination phenotype with \textit{papG} allele III independent of host species and demonstrated that a specificity for OP1 erythrocytes differentiated the NS phenotype associated with \textit{papG} allele III from that associated with Sfa.

Further phenotypic differentiation of strains containing \textit{papG} allele III was obtained by using \( \text{P}^k \) and \( \text{p} \) phenotype human erythrocytes, which lack the extended digalactoside-containing receptors preferred by PapG variant III. With these rare phenotype erythrocytes, strains containing \textit{papG} allele III (whether from dogs or humans) yielded trace or absent MRHA. In contrast, strains containing \textit{papG} allele II (whether from dogs or humans) exhibited MRHA that was robust (\( \text{P}^k \) cells) or of only moderately reduced intensity (\( \text{p} \) cells) (Table 2). The Sfa and Dr control strains agglutinated both \( \text{P}^k \) and \( \text{p} \) erythrocytes equivalently to A1P1 and OP1 cells (Table 2). Taken together, the agglutination results indicated that the human and canine strains containing \textit{papG} allele III exhibited indistinguishable digalactoside-specific agglutination phenotypes that collectively were distinct from those exhibited by canine and human strains containing \textit{papG} allele II and from those conferred by non-P MR adhesins.

Canine versus human ExPEC: PapG variant III sequence comparisons. Despite the similar agglutination phenotypes exhibited by human-derived strains versus canine-derived strains containing \textit{papG} allele III, it was hypothesized that these two groups still might differ with respect to the peptide sequences of their respective allele III PapG variants and hence might exhibit subtle host-specific differences in the precise receptor specificities of their P fimbriae. To test this hypothesis, the nucleotide sequence was determined for full-length \textit{papG} amplicons from two allele III-containing canine urine isolates from the present study, i.e., strains 1R (serogroup O4) and 4U (serogroup O6), and from two \textit{papG} allele III-containing human bacteremia isolates, i.e., strains CP9 (serogroup O4) (24, 53) and U7 (serogroup O6) (26). Predicted PapG peptides corresponding with these \textit{papG} sequences were compared with published sequences for PapG variant III from human ExPEC strain J96 (46, 70), canine ExPEC strain 1442 (5, 46), human asymptomatic bacteriuria isolate 83972 (11), and porcine septicemia isolate 4787 (10), yielding a total of four human, three canine, and one porcine representatives of the PapG variant III peptide.

The eight predicted PapG variant III peptides were highly homologous overall, exhibiting 91.7% identity and 51.1% strong similarity (Fig. 3A). At every position in the aligned peptides at which a polymorphism was detected there was sequence identity between at least one human and one canine PapG variant III peptide (Fig. 3A). In a similarity dendrogram, the eight
PapG variant III peptides were grouped as three closely related clusters, each of which contained representatives from two different host species (Fig. 4A). Human and canine variants were intermixed in two of the three clusters (Fig. 4A). The aggregate PapG variant III cluster was well removed from the clusters containing, respectively, the variant I and variant II PapG peptides (Fig. 4A). Taken together, these data indicate that versions of PapG variant III from dogs versus from humans do not segregate according to host species even at the peptide level.

Other pap elements. Each of the canine strains appeared to contain a complete copy of pap, as evidenced by PCR detection in each strain of all four pap regions, i.e., papAH, papC, papEF, and papG. The single MRHA-negative strain had no detectable abnormality of its pap genotype to account for its altered phenotype (Table 2).

**papA alleles.** Although all 16 canine strains were PCR positive for papAH, only seven had a PCR-detectable papA allele corresponding with 1 of the 11 serologically defined F types of P fimbriae, i.e., F7-1, F7-2, and F8-F16 (Table 1). The strains were next tested by using a primer specific for a novel papA allele (F48) that was recently discovered in human urosepsis isolate U7 (27). With the F48-specific primer, all nine of the canine strains that were F PCR negative in the initial testing yielded an amplicon of the size expected for the F48 PCR product, evidence that they contained the F48 papA variant.

### TABLE 2. Agglutination patterns of human and canine pap-positive strains for erythrocytes and P latex beads

<table>
<thead>
<tr>
<th>Source</th>
<th>Adhesin (no. of strains)</th>
<th>MRHA of human RBCs&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Neuraminidase treatment&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Agglutination of P beads&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
<td></td>
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<td>A&lt;sub&gt;p&lt;/sub&gt;P&lt;sub&gt;r&lt;/sub&gt;, and/or OP&lt;sub&gt;r&lt;/sub&gt;, RBCs</td>
<td>A&lt;sub&gt;p&lt;/sub&gt; OP&lt;sub&gt;r&lt;/sub&gt; RBCs</td>
<td>P&lt;sub&gt;o&lt;/sub&gt; or p RBCs</td>
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<tr>
<td></td>
<td></td>
<td>PBS or BSA</td>
<td>Pigeon egg white</td>
<td>Fetus</td>
</tr>
<tr>
<td>Human</td>
<td>papG allele II (2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>papG allele III (5)</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>Sfa (1)</td>
<td>+</td>
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<td></td>
<td>Dr (1)</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Canine</td>
<td>papG allele II (2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>papG allele III (13)</td>
<td>+</td>
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</table>

<sup>a</sup> For MRHA of A<sub>p</sub>P<sub>r</sub>, and OP<sub>r</sub>, RBCs, symbols are as follows: +, MRHA was as intense as positive control; −, MRHA was <2 intensity levels weaker than positive control (including absent MRHA). For MRHA of P or p RBCs, symbols are as follows: +, MRHA was as intense as positive control; (+), MRHA was 0 to 2 intensity levels weaker than positive control; −, absent MRHA. RBCs, red blood cells.

<sup>b</sup> Human-source control strains included IA2 and HB101/pDC1 (papG allele II); BOS055, BOS800, BOS117, and HB101/pFK102 (papG allele III); E1U968-298 (Sfa); and C1845 (Dr adhesins). Agglutination-positive canine strains (from present study) included 2U and 2R (papG allele II only); 1U, 1R, 3U-6R, 8U, and 8R (papG allele III only), and 7R (papG alleles II plus III, plus allele I variant). Strain 7U (papG alleles II + III, plus allele I variant) was agglutination negative.

<sup>c</sup> For agglutination of P beads, presence (+) and absence (−) of agglutination is indicated.
This was confirmed by full-length DNA sequencing of the papAH amplicon from one representative of each of the F48-positive pairs of urine and rectal strains (i.e., strains 2U, 4U, 5U, and 8U), plus from the sole unpaired F48-positive strain (strain 6R). The corresponding predicted PapA peptide sequences were highly homologous with one another and with the original F48 PapA sequence from (human) source strain U7 (Fig. 3B) but were distant from the other 11 PapA alleles (Fig. 4B). At each of the 17 positions in the F48 PapA peptide at which a polymorphism was detected among the six variants analyzed, the human-derived F48 variant exhibited identity with two or more of the five canine variants, and at only one position (residue 154) was the human-derived variant not identical to the majority of the canine variants (Fig. 3B). The F48 peptide from human isolate U7 was actually more similar overall to two of the canine F48 peptides than were the other three canine F48 variants (Fig. 4B).

Comparison of newly determined papA alleles with previously determined pilin sizes revealed close associations between the F48 papA allele and an 18.7-kDa pilin and between the F10 papA allele and a 19.7 kDa pilin (Table 1). The F48 papA variant accounted for all strains in the O6 cluster (clonal group C), but also occurred in the two outlier strains containing papG allele II only (clonal group D). In contrast, the F10 papA variant was limited to the O4 cluster (clonal group A) and occurred in all O4 strains, one of which also had the F7-2 papA allele (Table 1). These data provided phenotypic validation of the genetic differences detected by the F PCR assay, demonstrated the presence among canine ExPEC strains of papA alleles that are also prevalent among human ExPEC strains (50), and suggested both a phylogenetic distribution of papA alleles and horizontal mobility of the F48 papA variant.

Non-pap virulence factor (VF) genes. In addition to pap, the canine strains contained multiple other virulence-associated genes characteristic of human ExPEC strains (13). These included sfa/focDE (common to S fimbriae and F1C fimbriae), sfaS (S fimbrial adhesin), fyuA (yersiniabactin), cnf1, hlyA, cdtB (encoding cytolethal distending toxin), kpsMT-II and -III (group II and group III polysaccharide capsule synthesis), rfc (O4 lipopolysaccharide synthesis), traT (serum resistance), ompT, and a marker for a pathogenicity-associated island (PAI) from human ExPEC strain CFT073 (13). Several of these genes exhibited a clear-cut clonal distribution (Fig. 2; Table 1), as has been demonstrated also among human urosepsis isolates (26).

Commonality between canine and human ExPEC. To determine whether any overlap could be identified at the individual clone level between canine and human ExPEC strains, the 16 canine strains from the present study were compared with 75 previously described human urosepsis isolates with respect to O antigen, papG alleles, papA alleles, and genotype for multiple non-pap VFs (as shown in Table 1). One perfect match was found: human blood isolate U7 (O6:K?:HN), the source strain for the F48 papA allele (13, 27), matched canine strains 4U and 4R with respect to all of the characteristics analyzed. PFGE analysis showed human isolate U7 to be almost indistinguishable from these paired canine isolates, meeting criteria for "probably clonally related" according to Tenover et al. (65). Human isolate U7 actually resembled canine strains 4U and 4R more closely than did any of the other canine isolates, including even members of the same O6 clonal group (Fig. 5).

FIG. 3. Peptide polymorphisms for versions of PapG variant III (A) and the F48 PapA variant (B) from animal and human E. coli isolates. Comparisons included only predicted mature peptides. Alignments were by CLUSTAL-W (66). Only polymorphic positions are shown. Dashes indicate identity, and asterisks indicate gaps. Numbers above sequences indicate residue number (counting from the first residue of the mature peptide). Symbols: colons, highly similar amino acid substitutions; periods, slightly similar substitutions; blanks, differences. The single-letter amino acid code used is as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr. (A) For PapG variant III, the version from strain J96 was used as the comparator for versions from three canine isolates, three human isolates, and one porcine isolate. (B) For the F48 PapA variant, the version from index strain U7 (a human urosepsis isolate) was used as the comparator for versions from each of the five unique F48-positive canine isolates from the present study (strains 2U, 4U, 5U, 6R, and 8U).
Strains 4U, 4R, and U7 were subsequently found to be indistinguishable from one another by RAPD fingerprinting (not shown).

**DISCUSSION**

In the present study we have analyzed genotypic and phenotypic properties of ExPEC strains isolated from dogs and humans. Our results indicate that *papG* allele III, which predominates among human cystitis isolates, also predominates among *pap*-positive canine UTI isolates. Our data also show that (i) *papG* variant III sequences from dogs and humans are largely indistinguishable, (ii) canine ExPEC strains exhibit many non-*pap* virulence genes characteristic of human ExPEC strains, and (iii) even with highly sensitive typing methods, certain canine UTI isolates are essentially indistinguishable from certain human ExPEC strains, providing evidence that there are *papG* allele III-containing “crossover clones” of *E. coli* whose members are capable of causing UTI in both canine and human hosts.

Previous investigations of the MR adhesins of canine ExPEC strains led to conflicting conclusions regarding the relationship between canine and human ExPEC strains. It has been reported that canine ExPEC strains typically do not express P fimbriae and thus are fundamentally different from human ExPEC strains (74), that they express P fimbriae and hence are similar to human ExPEC strains (71), and that they express variant forms of P fimbriae that are dissimilar to the P fimbriae of most human ExPEC strains (5, 7, 36, 37, 41, 56, 57), but may be present in some human isolates (36, 37, 41, 56, 57). These investigations were done before assays for the three *papG* alleles were generally available (12, 16), before the epidemiological association of *papG* allele III with human cystitis had been demonstrated (12, 23, 59), and before it was known that receptors for *papG* variant III are not confined to non-human hosts (5, 7, 46, 62, 63) or to humans of the A blood phenotype (36, 37, 56) but are present generally in humans, including within the human urogenital tract (e.g., as sialosyl-galactosyl-globoside) (31, 32, 60, 64). These more recent developments, together with the results of the present study, clarify several points. They provide an explanation for the characteristic NS phenotype of *pap*-positive canine ExPEC strains (41), which is probably due to desialation by neuraminidase of sialosyl-galactosyl-globoside, the major receptor on human erythrocytes for the variant III *papG* adhesin (32, 56, 60, 64). They account for the seemingly discrepant MRHA results reported for canine ExPEC strains by various investigators (5, 7, 56, 57, 62, 71, 74), since the observed phenotypic variability is consistent with conflicting published data regarding *papG* variant III (15, 28, 32, 36, 62). They explain the nonreactivity of canine ExPEC strains with digalactoside-coated latex beads (41, 71) and with CTH-coated equine erythrocytes (74), which lack the extended structures preferred as receptors by PapG variant III (36, 60, 64). Finally, they contradict the assumption that canine ExPEC strains have an adhesin distinct from that of human ExPEC strains, one which recognizes a receptor not present in humans (5, 7, 46, 62) or present in only a minority of humans (36, 37, 56).

The results presented here confirm previous reports regarding the high prevalence of *pap, hly, sfa/foc*, and *cnf* among canine ExPEC strains (5, 7, 57, 71, 74, 77) and add *sfaS*, *fmuA*, *cdB*, *kpsMT* II and -III, *rfe, traT*, *ompT*, and the PAI marker from CFT073 to the list of virulence-associated genes present in both canine and human ExPEC strains (9, 26, 55). It is of interest that whereas the aerobactin system was not present in any of the canine strains, which is consistent with the low

FIG. 4. Dendrogram of PapG (A) and PapA (B) peptide sequences. Cluster analysis of predicted mature peptides was by the NJ method (54). (A) Representatives of *papG* variants I, II, and III are compared. The allele III variants include three from dogs (1442, 1U, and 4R), four from humans (J96, CP9, U7, and 83972), and one from porcine septicemia isolate 4787. A novel allele I variant identified in canine strain 7U (also shown in bold) will be reported separately. Sequences for CP9, U7, 1U, and 4R (all variant III) and 7U (variant I) were newly determined in the present study; sequences for the other *papG* variants were as previously published. (B) Five canine F48 PapA variants (from canine strains 2U, 4U, 5U, 6R, and 8U) are compared with a representative of each of the 11 serologically defined F types of P fimbriae, plus recently discovered PapA variants F40 and F48 (asterisks) (27).
prevalence reported by others (71, 77), the yersiniabactin siderophore system (fyuA), which has recently been associated with human septicemic E. coli (26, 55), was present in all but two of the strains. In addition, the presence of the PAI marker in most of the canine strains suggests that these strains have inherited a block of urovirulence genes similar to that present in archetypal human ExPEC strain CFT073 (9, 26, 30) and hence likely share with CFT073 and other human ExPEC strains many additional as-yet-unrecognized virulence genes.

Our demonstration of the near-identity of two canine strains (paired urine and rectal isolates from one canine host) and a blood isolate from an adult human with urosepsis confirms the existence of crossover canine-human ExPEC clones. These three strains shared the O6 antigen and had papG allele III, the F48 allele of papA, a uniform extended virulence genotype, and genomic fingerprints that were nearly (PFGE) or completely (RAPD) indistinguishable. Although previous studies have demonstrated overlap between canine and human ExPEC strains with respect to the O serogroup (5, 41, 71, 72, 74, 76), O:K serotype (57), allozyme type (72), or pap, hly, and IS5 RFLP patterns (41), PFGE is more discriminating than any of these typing methods (1, 40, 47, 48). Consequently, the present study provides the best evidence to date of commonality at the individual clone level between canine and human ExPEC strains, albeit with only a single example.

That only one of the 75 human isolates screened could be matched precisely to a canine strain does not necessarily mean that commonality between canine and human ExPEC strains is rare. The human strain collection screened in the present study, which was selected because its members had already been extensively characterized (14, 18, 20, 21, 26), consisted of blood isolates from patients with urosepsis and included only five papG allele-III containing strains. It seems probable that human strains that match canine ExPEC strains would be found more frequently within collections of human cystitis isolates, which exhibit a higher prevalence of papG allele III than do bacteremia isolates (12, 14, 17, 19, 23, 51). Consistent with this prediction, we have recently discovered that the (papG allele III-containing) O6:F48 clonal group that accounted for most of the canine isolates in the present study is one of the most prevalent clonal groups also among urine isolates from women with acute cystitis (unpublished data).

Seven of the eight canine urine isolates from the present

![FIG. 5. SmaI PFGE fingerprints from 14 canine urine and rectal isolates, plus one human urosepsis isolate, of E. coli. For the canine isolates, strain names (dog number, urine versus rectal source) correspond with those shown in Table 1. Highly similar strains 4U (canine), 4R (canine), and U7 (human) are shown in bold. (Canine isolates 2U and 2R [not shown] were indistinguishable from one another and different from all other strains.)](http://iai.asm.org/)

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study were essentially indistinguishable from the corresponding rectal isolate from the same host, but were distinct from urine and rectal isolates from other canine hosts, with respect to RAPD and PFGE fingerprints and to profiles for multiple virulence-associated genes. This provides strong added support for the hypothesis that canine ExPEC strains (41), like human ExPEC strains (8), typically originate from within the host’s own fecal flora and are the predominant fecal E. coli strains at the time of UTI. Other data suggest that even healthy dogs may commonly be fecally colonized with ExPEC strains (73, 77). It is likely that through contact with dogs or their excreta, many humans are routinely exposed (on a macroscopic or microscopic level) to elements of the canine fecal flora. Further study of the health implications for humans of such exposures is needed. If pets and pet feces are found to represent significant sources of pathogens or antibiotic resistance elements, interventions that could directly block transmission of fecal bacteria from animals to humans or that could reduce the prevalence and intensity of colonization of pets with pathogenic and/or antimicrobial-resistant E. coli strains conceivably could offer important new measures to prevent human disease.

Summary. In the present study, papG allele III was found to be the predominant papG allele among E. coli isolates from dogs with UTI, to account for the atypical agglutination pattern of these canine ExPEC isolates and to be genetically indistinguishable among canine versus human ExPEC strains. Multiple virulence genes associated with human ExPEC isolates were identified in pap-positive canine E. coli isolates. One pair of canine urine and rectal isolates was nearly indistinguishable from a human urosepsis isolate with respect to all characteristics studied, including PFGE and RAPD fingerprints, hence presumably represented essentially the same clone. The rectal-urine hypothesis of canine UTI pathogenesis was confirmed by RAPD and PFGE fingerprinting. These findings implicate dogs, and specifically canine feces, as potential reservoirs of E. coli strains with extraintestinal pathogenic capability for humans.

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