Canine Feces as a Reservoir of Extraintestinal Pathogenic
Escherichia coli

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To test the canine reservoir hypothesis of extraintestinal pathogenic Escherichia coli (ExPEC), 63 environmental canine fecal deposits were evaluated for the presence of ExPEC by a combination of selective culturing, extended virulence genotyping, hemagglutination testing, O serotyping, and PCR-based phylotyping. Overall, 30% of canine fecal samples (56% of those that yielded viable E. coli) contained papG-positive E. coli, usually as the predominant E. coli strain and always possessing papG allele III (which encodes variant III of the P-fimbrial adhesin molecule PapG). Multiple other virulence-associated genes typical of human ExPEC were prevalent among the canine fecal isolates. According to serotyping, virulence genotyping, and random amplified polymorphic DNA analysis, over 50% of papG-positive fecal E. coli could be directly correlated with specific human clinical isolates from patients with cystitis, pyelonephritis, bacteremia, or meningitis, including archetypal human ExPEC strains 536, CP9, and RS218. Five canine fecal isolates and (clonally related) archetypal human pyelonephritis isolate 536 were found to share a novel allele of papA (which encodes the P-fimbrial structural subunit PapA). These data confirm that ExPEC representing known virulent clones are highly prevalent in canine feces, which consequently may provide a reservoir of ExPEC for acquisition by humans.

Dogs have been proposed as a possible reservoir of the virulent Escherichia coli strains that cause extraintestinal infections in humans (extraintestinal pathogenic E. coli [ExPEC]) (2, 33, 53, 57). This hypothesis is based on several lines of evidence, including (i) the documented similarities between certain canine and human urinary tract infection (UTI) isolates of E. coli with respect to virulence factors (VFs), O antigens, and evolutionary lineage (33, 53–55, 57), (ii) the observation that in dogs with UTI the infecting E. coli strain often derives immediately from the host’s own fecal flora (33), and (iii) the high prevalence of UTI-associated VFs among canine fecal E. coli isolates (57). However, doubts regarding the validity of the canine reservoir hypothesis have persisted (2) because of the differences noted in some studies between canine and human ExPEC isolates with respect to adherence phenotypes (8, 33, 48, 55) and surface antigens (48, 56), which presumably reflect clonal relationships.

The ostensibly atypical agglutination phenotypes of canine UTI isolates were recently shown to be due to expression by canine strains of papG allele III, which encodes a variant of the P-fimbrial adhesin molecule PapG that is now known to be epidemiologically associated with human cystitis (23). The agglutination phenotypes of strains that expressed papG allele III were found to be indistinguishable among canine and human isolates (23). These findings addressed the first major argument against considering canine-derived ExPEC isolates as potential human pathogens. In addition, clonal overlap was documented between human and canine ExPEC isolates, which confirmed that dogs sometimes are colonized with the same ExPEC types as cause extraintestinal infections in humans (21, 23). This addressed the second major argument against the canine reservoir hypothesis.

The canine reservoir hypothesis is important because of its potential implications for the development of new preventive measures against UTI and other extraintestinal infections in humans. In the present study, we sought to further evaluate this hypothesis by determining the prevalence in canine feces of E. coli strains exhibiting VFs characteristic of human ExPEC and by searching for evidence of clonal commonality between canine fecal E. coli and E. coli clinical isolates from humans.

MATERIALS AND METHODS
Canine fecal samples. Sixty-three putative fecal deposits of putative canine origin (as determined by appearance and location; hereafter referred to as canine fecal samples) were collected from alongside municipal sidewalks in a predominantly residential neighborhood of St. Paul, Minn., during April and May 1996 and 1997. All available canine fecal samples from the area surveyed were collected except for the restriction (imposed to maximize diversity) that no more than one sample could be collected per 40 ft of sidewalk. When multiple samples were available within one 40-ft zone, preference was given to the sample that appeared freshest. Approximately 60 linear city blocks were screened to obtain the 63 samples. Fecal samples were sealed individually in plastic food storage bags at the time of collection and were refrigerated until processed. In the laboratory, samples were incubated overnight at 37°C in Luria broth (34), which was then plated to MacConkey’s agar. From plates that yielded isolated colonies, three individual lactose-positive colonies with a colonial morphology consistent with E. coli (if available) were picked at random, tested by Gram stain and indole production to confirm their identity as putative E. coli, and frozen at −70°C in 15% glycerol. Ambiguous identifications were further evaluated by using the API-20E system (bioMérieux). From all cultures that yielded growth on MacConkey’s agar (whether or not E. coli was evident), a sweep of the mixed growth from the inoculum zone also was frozen at −70°C in 15% glycerol.

Control strains. Human clinical isolates that were compared with selected canine fecal isolates included urosepsis isolates U7 (O6:K2:H7:F48) and 2H25 (O18:K1:H7:F10) (29), bacteremia isolates BOS035 (O6:F48) (18) and CP9 (O4:K54:H5:F13,F14) (26), cystitis isolates 466 (O6:F48) (25) and U64 (O18:K1:H7:F10) (32), neonatal meningitis isolate RS218 (O18:K1:H7:F10) (4),...
and archetypal ExPEC strain 536 (O6:K15:H31) (9). Strains from the Escherichia coli Reference (ECOR) collection which represent each of the four major phylogenetic groups of E. coli (A, B1, B2, and D), plus the nonaligned strains, as defined by multilocus enzyme electrophoresis (10), were included as phylogenetic controls.

Amplification fingerprinting. Random amplified polymorphic DNA (RAPD) fingerprints were generated using arbitrary decamer oligonucleotide primers as previously described (23). For the three E. coli colonies from each canine fecal sample that contained E. coli, RAPD fingerprints (from two different primers, used separately) were compared visually to determine the number of unique predominant strains present in each sample, with only one representative of each predominant strain processed further. For the phylogenetic analysis, composite RAPD fingerprints were constructed for all putative unique canine fecal isolates and for the human and ECOR control strains by digitally combining in a head-to-tail fashion two to five different newly generated single-primer RAPD fingerprints for each isolate (23). Pearson’s correlation coefficient analysis of all pairwise comparisons between different composite fingerprints (which was done based on analog densitometric scans of gel tracks, without definition of discrete bands) was used to generate similarity matrices. Dendrograms were then constructed according to the unpaired group method with averaging (UPGMA) (49) by using the application Molecular Analyst (Bio-Rad, Hercules, Calif.).

Hemagglutination. Mannose-resistant hemagglutination (MRHA) was assessed using human A, P, and sheep erythrocytes in microscope slide assays done at 4°C with microscopic detection, as previously described (15, 23), without reference to adhesin genotyping results. MRHA intensity was graded semiquantitatively on a four-point scale, from 0 (absent) to 4+ (maximally intense, with most erythrocytes aggregated into large clumps). Pigeon egg white was used as a digalactoside-containing inhibitor of P-fimbrial adherence (15, 24). A decrement in MRHA intensity by ≥3 intensity levels in the presence of pigeon egg white was interpreted as P-pattern MRHA (15, 23). Lesser degrees of inhibition were interpreted as non-P MRHA (15).

Detection and recovery of occult papG-positive canine fecal strains. A representative of each unique E. coli genotype from each canine fecal sample that yielded isolated E. coli colonies, plus the mixed growth sample from each canine fecal culture, was tested for the three alleles of papG using an established multiplex PCR assay (17). Mixed samples that yielded a positive papG PCR result that could not be accounted for by any of the initially analyzed unique E. coli genotypes from the corresponding sample were processed further to recover the presumed occult papG-positive strain. Selective hemadsorption to human or sheep erythrocytes was used to trap P-fimbriated bacteria within mixed samples, thereby allowing them to be separated from non-P-fimbriated bacteria by differential centrifugation and washings, as previously described (16). MRHA-positive colonies from selective hemadsorption platings on blood agar were tested for the papG alleles. Several papG-positive colonies from each sample were then compared by RAPD fingerprinting with the predominant genotype(s) from the same sample. A representative of each newly extracted unique genotype from each sample was subsequently processed in parallel with the initially isolated predominant strains.

Virulence genotypes. All unique canine fecal isolates and all control strains were tested for 31 putative virulence genes of E. coli using a multiplex PCR assay, with the addition of DNA probe hybridization for several genes (fnbA, fimH, hly, cnf1, and kpsMT II), as previously described (27, 29). Selected strains that were positive by PCR for papAH were further tested for the 12 known F-specific alleles of papG using an established multiplex PCR assay (30). For strains that were papG-positive but F PCR negative, the papAH PCR product was directly sequenced as previously described (30). The predicted PapA peptides were then aligned with the 12 known PapA variants using CLUSTAL W (50), and a similar dendrogram was inferred according to the neighbor-joining method by using the application MEGA (31).

Nicotinamide auxotrophy. O157:K1 strains were assessed for their growth requirement for supplemental nicotinamide at 30 and 39°C on minimal medium agar containing glucose as previously described (1, 32). Nicotinamide auxotrophy at both 30 and 39°C was interpreted as evidence of membership in the outer membrane pattern (OMP) 6 subtype of the O157:K1 clone, whereas nicotinamide auxotrophy at 39 but not 30°C was interpreted as evidence of membership in the OMP 9 subtype of the O157:K1 clone (1).

Serotyping. Detection of O antigens was done by the Exscherichia coli Reference Center (University Park, Pa.). O antigens classically associated with extraintestinal infections in humans (O1, O2, O4, O6, O7, O8, O16, O18, O25, and O75) were regarded as ExPEC-associated O antigens (14).

Statistical methods. Comparisons of proportions were tested using Fisher’s exact test. Comparisons of the prevalence of different traits in the same population were tested using McNemar’s test (7). The threshold for statistical significance was P < 0.05.

Nucleotide sequence accession numbers. papG sequences determined in this study were deposited in GenBank under accession numbers AF237477 (strain 536) and AF255005 to AF255009 (strains 23e, 14e, 1, 2, and 5a, respectively).

RESULTS

Recovery of papG-positive E. coli from canine fecal samples. Fifty-nine (94%) of the 63 canine fecal samples yielded growth on MacConkey’s agar after an initial broth enrichment step. Of these culture-positive samples, 34 (58%) yielded isolated colonies of E. coli (i.e., were E. coli-positive samples), whereas 25 (42%) yielded only non-E. coli gram-negative bacilli, predominantly presumptive Proteus and Klebsiella-Enterobacter spp. RAPD fingerprinting of three arbitrarily selected E. coli colonies from each of the 34 E. coli-positive samples revealed a single genotype in 27 samples and two distinct genotypes in seven samples, giving a total of 41 putative predominant fecal E. coli strains.

PCR analysis of the mixed bacterial growth from MacConkey’s agar plates confirmed the presence of one or more papG alleles in 19 (32%) of the 59 culture-positive fecal samples. papG positivity was limited to the 34 E. coli-positive samples (56% papG positive, versus 0% for other samples; P < 0.001). In addition to papG allele III, which was present in every papG-positive sample, one sample each also had papG allele I or papG allele II (for prevalence of papG allele III versus allele I or allele II; P < 0.01, McNemar’s test). PCR analysis of the 41 individual predominant E. coli strains identified as papG positive 13 of these strains, each of which was associated with a papG-positive mixed sample. Each papG-positive predominant strain exhibited the same papG allele configuration as did the corresponding mixed sample, i.e., allele III only (n = 12) or alleles II plus III (n = 1). This left six mixed samples for which the positive papG result could not be accounted for by a predominant strain from that sample. Thus, in these samples papG positivity presumably was due to an occult papG-positive strain that was present in the mixed sample but not among the three isolated colonies initially picked for individual analysis.

From each of these six mixed samples, papG-positive isolates were successfully extracted by a combination of selective hemadsorption enrichment and screening for hemolysin on blood agar. For each sample, the multiple papG-positive colonies that were recovered yielded a uniform RAPD genomic fingerprint, indicating that the isolates from a given sample were all replicates of a single strain. As with the papG-positive predominant strains, in each instance the papG-positive occult strain exhibited a papG allele configuration consistent with that of the corresponding mixed sample, i.e., papG allele III only (n = 5) and papG alleles I plus III (n = 1). Five of the six occult papG-positive strains had RAPD fingerprints distinct from those of the predominant strain(s) from the corresponding sample, evidence that the papG-positive strain represented a distinct (unrelated) strain. In contrast, one occult papG-positive strain (strain 25e) was indistinguishable by RAPD analysis from the corresponding sample’s (single) papG-negative predominant strain (strain 25a), evidence of a clonal relationship between these strains despite their differing papG genotypes.

MRHA phenotypes. All 19 papG-positive canine fecal strains exhibited P-pattern MRHA. One papG-negative strain exhib-
prints from the 47 canine fecal E. coli
ence genotype. Cluster analysis of composite RAPD finger-
were MRHA negative. The remaining 27 papG-negative strains
ited non-P MRHA. The remaining 27 papG-negative strains
were MRHA negative.

**Population structure in relation to O serogroup and virulence genotype.** Cluster analysis of composite RAPD finger-
prints from the 47 canine fecal E. coli isolates revealed two
major phylogenetic clusters (Fig. 1). Of the 14 isolates that
constituted the smaller of these clusters (cluster 1), few exhib-
ited ExPEC-associated O antigens or contained many viru-
ulence genes other than fimH (Table 1). In contrast, most of
the 27 isolates that constituted the larger cluster (cluster 2) ex-
pressed ExPEC-associated O antigens and contained multiple
virulence genes, including various combinations of pap ele-
ments, sfa/foc, sfaS, focG, iha, hlyA, cnf1, fyuA, iroN, group II
or group III kpsMT variants, ibeA, and the PAI (pathogenicity
island) marker from strain CFT073 (Table 1). Of the 12 rec-
ognized papA alleles, the F10, F12, F13, F14, and F48 variants
were detected and were concentrated in phylogenetic cluster 2.
Two strains in cluster 2 each had two different papA alleles;
both strains also had two different papG alleles, consistent with
the presence of two complete pap operons (Table 1).

The paucity of ExPEC-associated O antigens and VF genes
in cluster 1 suggested that this cluster might correspond with
phylogenetic groups A, B1, and/or nonaligned. In contrast, the
abundance of ExPEC-associated O antigens and virulence
genes in cluster 2 suggested that this cluster might correspond
with virulence-associated phylogenetic group B2. These hy-
potheses were confirmed by comparative RAPD analysis of
representative members of clusters 1 and 2 and of relevant
ECOR control strains (e.g., Table 2).

**Comparison of canine fecal isolates with human clinical
ExPEC isolates.** Inspection of O antigens and virulence geno-
types revealed striking similarities between certain canine fecal
isolates from cluster 2 (e.g., strains 30, 19, 12e, 11, and 20) and
selected human clinical isolates (Table 2). Consequently, these
five canine fecal isolates were compared directly with appro-
priate human clinical isolates and with ECOR control strains
in a third round of composite RAPD fingerprinting (Fig. 2 and
3). The 10 canine fecal isolates and the six human clinical
isolates clustered together with the group B2 ECOR control
strains, apart from the non-B2 ECOR strains (Fig. 3). Within
the B2 cluster, three subclusters corresponding with the three
serogroups analyzed, i.e., O6, O4, and O18 (subclusters A, B,
and C, respectively), were resolved (Fig. 3). Within each of
these subclusters, human and canine isolates were essentially
indistinguishable (Fig. 3), evidence of commonality at the
genomic level as well as with respect to O antigen and viru-
ulence genotype. Nicotinamide auxotrophy testing of the human
and canine O18:K1 isolates showed them all to exhibit a re-
quirement for nicotinamide supplementation at both 30 and
39°C, consistent with membership in the OMP 6 subclone of E.
coli O18:K1:H7 (not shown).

The extensive virulence genotype similarities (Table 1) noted between O6:F48 strain 25e (an outlier in Fig. 1) and the
two O6:F48 strains from cluster 2 (Fig. 1) suggested the pos-
sibility of genomic similarities that may have been missed in
the initial round of composite RAPD fingerprinting. Conse-
quently, strains 25e and 25a (the papG-negative predominant
strain from the same fecal sample as 25e) were subjected to
repeat composite RAPD fingerprinting along with the two
O6:F48 isolates from cluster 2 (strains 19 and 30) and relevant
ECOR controls. The four O6 isolates now yielded essentially
indistinguishable RAPD fingerprints, and all clearly fell within
phylogenetic group B2 (not shown). This confirmed the two
putative outlier O6 isolates (25a and 25e) as actually belonging
with the other canine O6:F48 strains as members of cluster 2
(Fig. 1), hence also as closely related to the O6:F48 human
clinical isolates (Table 2).

![Diagram of phylogenetic relationships among 63 canine fecal isolates according to RAPD analysis.](http://iai.asm.org/)
A novel PapA variant and the 536-like clonal group. Five of the canine fecal strains (strains 1, 2, 5a, 14e, and 23e) were PCR positive for *papA* but were negative in the F PCR assay for a recognized *papA* allele (Table 1). All five strains were from cluster 2, expressed the O6 antigen, and exhibited a fairly homogeneous virulence genotype (Table 1), evidence suggesting that they might represent a clonal group containing a novel variant of *papA*. To test this hypothesis, we determined *papA* sequence for these five strains and compared the predicted PapA peptides with known PapA variants. In a similarity dendrogram the five canine PapA variants clustered together, well removed from the 12 control PapA sequences. However, they were closely related to PapA from archetypal human ExPEC strain 536 (O6:K15:H31), the sequence of which we had recently determined after finding *pap*-positive strain 536 to be PCR negative for the 12 known *papA* alleles (Fig. 4). Comparative RAPD fingerprints as generated in parallel for these five O6 canine isolates and strain 536 showed that all six strains shared a common genomic background (Fig. 5). Comparative virulence genotyping revealed extensive additional similarities between these strains (Table 2). This confirmed that the five canine fecal isolates belong to a clonal group that includes
TABLE 2. Comparative characteristics of selected canine fecal from this study and human clinical isolates

| Strain group | Host | Source | Clonal type | kpnMT | papG | fyuA | iroN | fimH | hlyA | cnf1 | iha | focG | focS | kpsMT | Clonal PAI | cfa | ipaA | ipaE | mgt | mei | sat | sfa | sfaS | saa | sdr | slgA | ssp | yqf | yqf | yst | ysc | yscA | yscB | yscC | yscD | yscE |
|--------------|------|--------|-------------|-------|------|------|------|------|------|------|-----|------|------|-------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A            | Dog  | Fecal  | I, III      | +     | +    | -    | -    | -    | -    | -    | -    | -    | -    | -     | +          | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| B             | Dog  | Fecal  | III         | +     | +    | -    | -    | -    | -    | -    | -    | -    | -    | -     | +          | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| C             | Dog  | Fecal  | III         | +     | +    | -    | -    | -    | -    | -    | -    | -    | -    | -     | +          | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Human| Bacteremia | III         | +     | +    | -    | -    | -    | -    | -    | -    | -    | -    | -     | +          | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Human| Cystitis  | III         | +     | +    | -    | -    | -    | -    | -    | -    | -    | -    | -     | +          | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Human| Urethritis | III         | +     | +    | -    | -    | -    | -    | -    | -    | -    | -    | -     | +          | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
|               | Human| Pyelonephritis | III       | +     | +    | -    | -    | -    | -    | -    | -    | -    | -    | -     | +          | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |

* Isolates are sorted by clonal group (A, B, C, and “536,” i.e., strains similar to archetypal strain 536), and within each clonal group by host species. Definitions are as for Fig. 2. (All strains were negative for afa/dra, bmaE, nfaE, gafD, cdtB, iutA, and cvaC.)

In the present study we rigorously analyzed the virulence traits and phylogenetic background of E. coli isolates from canine fecal deposits and compared these strains with selected human clinical ExPEC isolates. We found that canine fecal E. coli commonly exhibit characteristics typical of human ExPEC and that most pap-positive canine fecal isolates can be directly correlated with clinical isolates from human patients with cystitis, pyelonephritis, bacteremia, or meningitis.

Our findings strongly support the canine reservoir hypothesis (21, 23, 33, 53, 54). In this study, papG-positive E. coli were recovered from 30% of all canine fecal deposits and from 56% of deposits from which viable E. coli were isolated. When present, papG-positive E. coli usually represented the predominant E. coli strain. Furthermore, over half of the papG-positive canine fecal E. coli isolates could be directly correlated with specific human clinical isolates representing known virulent clones of ExPEC which collectively have been implicated in all of the major E. coli extraintestinal infection syndromes.

These findings provide the best possible evidence short of actual human volunteer challenge studies that certain canine fecal strains are potential human pathogens. This is turn suggests that humans may acquire pathogenic bacteria through contact with canine feces, whether in the environment (as studied here) or by association with dogs (36, 52; W. B. Trenva, R. A. Hooper, C. Wray, G. A. Willshaw, T. Cheasty, and G. Domingue, letter, Vet. Rec. 20:400, 1996). Epidemiological studies are needed to determine whether such interspecies transfer of ExPEC occurs and, if it does, its frequency and clinical consequences for humans. Possible interventions that could be considered if dog-to-human transmission of ExPEC is found to contribute substantially to human disease might include wider use and stricter enforcement of municipal “pooper scooper” ordinances, heightened attention to personal hygiene vis-à-vis contact with dogs, and measures to reduce the prevalence or intensity of intestinal colonization with ExPEC among dogs.

This study illustrates the power for comparative strain analyses that is provided by the combination of extended virulence gene detection (including the alleles of papA and papG, plus sequence analysis of novel papA variants), PCR-based phylotyping, and O serotyping. Contributing to this study’s success in detecting matches between canine and human isolates was the availability of several collections of extensively characterized human-source ExPEC (18, 25, 29). It is probable that with a larger database of virulence genotypes, papA alleles, and other bacterial characteristics, additional matches would be found between canine and human isolates of E. coli. Compared with this study and a recent study from our laboratory (23), other studies of canine fecal or urinary E. coli isolates have examined a more limited range of VFs, have not combined VF analysis with phylogenetic analysis and surface antigen detection, or have not made as extensive comparisons with human clinical isolates (8, 33, 48, 53–57).

Consistent with a previous analysis of urine and fecal isolates from dogs with UTI (23), in the present study papG allele III...
was the predominant \( \text{pap}G \) allele among canine fecal isolates. This suggests that if humans do acquire ExPEC from dogs to any significant extent, this probably relates primarily to \( \text{pap}G \) allele III-containing strains, which are particularly common in the context of human cystitis (12, 25). Since strains that cause pyelonephritis and bacteremia in humans more commonly contain \( \text{pap}G \) allele II (11–13, 18, 38), for these strains other possible reservoirs will need to be investigated. Nonetheless, the participation of \( \text{pap}G \) allele III-containing ExPEC in diverse clinical syndromes in humans (Table 1) suggests that interventions directed toward a canine reservoir of such strains could have broad ranging clinical benefits. The apparent “generalist” pathogenic behavior of many ExPEC clones (e.g., Table 2) also indicates the inadequacy of restrictive designations for them such as uropathogenic \( E. \text{coli} \) (42).

The high prevalence of \( \text{ibe}A \) among the canine isolates (Table 1) was of interest, since this gene is associated with neonatal meningitis in humans (3). In this context, a curious subcluster within phylogenetic cluster 2 (i.e., strains 26, 5b, 23a, 7, and 8 [Fig. 1]) stood out by virtue of the uniform presence of \( \text{ibe}A \) and the high prevalence of \( \text{sfa} / \text{foc} \) despite the general absence of \( \text{pap} \) (Table 1). We have encountered a similar
and the three O6;F48 isolates, which in the initial phylotyping lence genotypes (Table 1), evidence that they probably actually dendrogram (Fig. 1) and were found to have identical viru-
genotypes, were placed as nearest neighbors in the phylotyping. For example, isolates 28a and 28c, which in the initial phylotyping. For example, isolates 28a and 28c, which in the initial phylotyping and fecal deposits such as are routinely encountered by human cross-contamination, etc.). Nonetheless, the study material bility of artifacts from environmental exposure (drying, cold, despite the efforts made to maximize diversity, and the possi-
bility between certain canine and human isolates from the O6; F48 clonal group was demonstrated by combinations of XbaI genomic macrorestriction analysis, multilocus enzyme electrophoresis, and extended virulence factor profiles, clear evidence of clonal overlap of pathogens between host species (21, 23). The present study extends these findings by showing that O6; F48 strains indistinguishable from certain human isolates are present also in environmental canine feces as well as among clinical isolates.

The O6;F48 clonal group that accounted for three of the canine fecal isolates from this study is prevalent among pappositive canine UTI isolates (23). It also accounts for 8% of diverse-source bacteremia isolates from adults (unpublished data) and for 8% of urine isolates from women with acute cystitis (20), as represented in this study by strains BOS035 and 466, respectively (Table 2). In other recent studies, commonality between certain canine and human isolates from the O6; F48 clonal group was demonstrated by combinations of XbaI genomic macrorestriction analysis, multilocus enzyme electrophoresis, and extended virulence factor profiles, clear evidence of clonal overlap of pathogens between host species (21, 23). The present study extends these findings by showing that O6; F48 strains indistinguishable from certain human isolates are present also in environmental canine fecal deposits.

Model human ExPEC strain 536 (O6;K15:H31), one of the strains in which PAIs were first discovered, has been exten-
sively investigated with respect to its virulence traits (5, 9, 35, 47). Commonality between strain 536 and five canine fecal isolates from this study was initially suggested by the serendip-
titous discovery of papA sequence homology among these strains. Comparisons of virulence genotypes and RAPD pro-
files confirmed the common clonal background of these strains. This discovery, which replicates findings from another recent study of human and animal isolates (21), nearly doubled the number of canine fecal isolates that could be correlated with human ExPEC.

In summary, we found that canine fecal E. coli strains commonly exhibit virulence traits and phylogenetic characteristics typical of human ExPEC. Most pap-positive canine fecal isolates could be directly correlated with known clinical isolates from human patients with cystitis, pyelonephritis, bacteremia, or neonatal meningitis. These findings strongly implicate canine feces (and, by extension, dogs) as a reservoir for humans of pathogenic E. coli, thus indicating a need for epidemiolog-

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**FIG. 5.** RAPD analysis of selected canine fecal isolates and human ExPEC strain 536. Fingerprints for canine isolates (lanes 2 to 6) and strain 536 (lane 7) were generated using RAPD primer 1281. (Primer 1283 gave similar results.) M, 100-bp molecular weight ladder (lanes 1 and 8). Sizes are indicated in base pairs.
ical studies to assess transmission rates and associated human health risks.

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