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Canine Feces as a Reservoir of Extraintestinal Pathogenic Escherichia coli

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Received 12 July 2000/Returned for modification 26 October 2000/Accepted 20 November 2000

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 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 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. 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;K+;H−;F48) and H25 (O18:K1:H7:F10) (29), bacteremia isolates BOS035 (O6;F48) (18) and CP9 (O4;K10;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, B, and sheep erythrocytes in microslide 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 5-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 non-P 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 by PCR for hlyA, cnf1, kpsMT, and with the addition of DNA probe hybridization for several genes (31). The presumed occult strains from the corresponding sample were processed further to recover E. coli by using the application Molecular Analyst (Bio-Rad, Hercules, Calif.). O antigens classically associated with ex-hemorrhagic E. coli 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 23c, 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 I and 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 and 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-
Cluster analysis of composite RAPD fingerprinting yielded 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 fingerprints 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 exhibited ExPEC-associated O antigens or contained many virulence genes other than fimH (Table 1). In contrast, most of the 27 isolates that constituted the larger cluster (cluster 2) expressed ExPEC-associated O antigens and contained multiple virulence genes, including various combinations of pap elements, 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 recognized 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 hypotheses 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 genotypes 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 appropriate 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 virulence genotype. Nicotinamide auxotrophy testing of the human and canine O18:K1 isolates showed them all to exhibit a requirement 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 possibility of genomic similarities that may have been missed in the initial round of composite RAPD fingerprinting. Consequently, 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).
A novel PapA variant and the 536-like clonal group. Five of the canine fecal strains (strains 1, 2, 5a, 14c, and 23e) were PCR positive for papaH 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 EsPEC strain 536 (O6:K15:H31), the sequence of which we had recently determined after finding papa-positive strain 536 to be PCR negative for the 12 known papa alleles (Fig. 4). Composite 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 and human clinical isolates

| Strain | Source | Host | O serotype | F type | Pap | P4 | Foc | FimH | HlyA | Cnf1 | FyuA | IroN | HtrA | KpnMT | KfaA | EaeA | gafD | mep | fyuA | iutA | cdtB | papG |
|--------|--------|------|------------|--------|-----|-----|-----|------|------|------|------|------|------|------|-------|------|------|------|------|------|------|------|------|------|------|
| A      | Fecal  | Dog  | 6          | IV     | +   | +   | +   | +    | +    | +    | +    | +    | +    | +    | +     | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| 30     | Bacteremia | Human | 6        | IV     | +   | +   | +   | +    | +    | +    | +    | +    | +    | +    | +     | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| B      | Fecal  | Dog  | 6          | IV     | +   | +   | +   | +    | +    | +    | +    | +    | +    | +    | +    | +     | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| 466    | Bacteremia | Human | 6        | IV     | +   | +   | +   | +    | +    | +    | +    | +    | +    | +    | +     | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| BOS035 | Bacteremia | Human | 6        | IV     | +   | +   | +   | +    | +    | +    | +    | +    | +    | +    | +     | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| 46b    | Cystitis | Human | 6        | IV     | +   | +   | +   | +    | +    | +    | +    | +    | +    | +    | +     | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| 20     | Bacteremia | Human | 6        | IV     | +   | +   | +   | +    | +    | +    | +    | +    | +    | +    | +     | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| 536    | Pyelonephritis | Human | 6    | IV     | +   | +   | +   | +    | +    | +    | +    | +    | +    | +    | +     | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |

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.)

DISCUSSION

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 fecal 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. Trevena, 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 analysis 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 phylotyping 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 papG allele among canine fecal isolates. This suggests that if humans do acquire ExPEC from dogs to any significant extent, this probably relates primarily to papG 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 papG allele II (11–13, 18, 38), for these strains other possible reservoirs will need to be investigated. Nonetheless, the participation of papG 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. coli (42).

The high prevalence of ibeA 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 ibeA and the high prevalence of sfa/foc despite the general absence of pap (Table 1). We have encountered a similar

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**FIG. 2.** Comparative RAPD analysis of selected canine fecal and human clinical isolates. Fingerprints were generated using RAPD primer 1247 for canine and human isolates (lanes 2 to 11 and 13 to 18) and ECOR control strains (identified as to phylogenetic group) (lanes 19 to 23 and 25 to 27). Clonal groupings correspond with those shown in Table 2. Lane M, 100-bp ladder. For canine isolates 11, 12e, 19, and 30, duplicate template DNA preparations (#1 and #2 for each strain) were amplified in parallel to assess same-day, same-strain reproducibility. Sizes are indicated in base pairs.

**FIG. 3.** Cluster analysis of composite RAPD fingerprints. Canine fecal and human clinical isolates are identified as to host (D, dog; H, human) and clinical source (B, bacteremia; CY, cystitis; F, fecal; NBM, neonatal bacterial meningitis). ECOR strains (shown in bold) are identified as to phylogenetic group. Dendrogram construction (by UP-GMA) was based on composite RAPD fingerprints from primers 1247 (Fig. 2), 1254, and 1281. Clusters A, B, and C (brackets) correspond with the clonal groups shown in Table 2 and Fig. 2. The marker lane cluster (MW), which extends to the 92.3% similarity level (dashed line), reflects the variability inherent in gel electrophoresis and image analysis, exclusive of PCR-related artifacts.

**FIG. 4.** Dendrogram of predicted PapA peptides. Predicted mature PapA peptides for the 12 established papA alleles (F7-1, F7-2, F8-F16, and F48) and for papA from canine fecal isolates 1, 2, 5a, 14e, and 23e and human ExPEC strain 536 (bold [this study]) were aligned by using CLUSTAL W. The dendrogram was inferred by the NJ method (46).
virulence genotype, often in association with the O83 antigen (as in strains 26, 5B, and 23), among cerebrospinal fluid isolates from human infants with meningitis (unpublished data), evidence suggesting the possibility presence of additional potential human pathogens within canine feces.

Based on their virulence genotype and nicotinamide auxotrophy pattern, the two (ibeA-positive) O18:K1 canine fecal isolates appeared to belong to the OMP 6 subclone of the O18:K1:H7 clone. In the United States, this subclone is associated with neonatal meningitis (1), cystitis in adult women (20, 32), and bacteremia in adults (reference 29 and unpublished data), as exemplified by control strains RS218, U64, and 2H25, respectively (Table 2). Whereas among O18:K1:H7 strains members of the OMP 6 subclone typically are aerobactin negative but possess PAIs containing pap, hly, and cnf (6, 37), members of the OMP9 subclone (which are prominent as agents of neonatal meningitis in Europe but are uncommon in North America) (1, 20, 51) are typically aerobactin positive but lack dog-associated VFs pap, hly, and cnf (1, 37).

Certain limitations of this study deserve comment. First, the study was geographically and temporally limited, making extrapolation to other locales and time periods uncertain. Second, the study included an initial broth amplification step, which conceivably could have altered the relative prevalence of the various E. coli strains present in each sample. Third, environmental canine fecal deposits rather than fresh fecal samples from individual canine hosts were studied. This introduces the possibility of multiple sampling of the same host despite the efforts made to maximize diversity, and the possibility of artifacts from environmental exposure (drying, cold, cross-contamination, etc.). Nonetheless, the study material does provide a valid representation of environmental canine fecal deposits such as are commonly encountered by human hosts irrespective of their pet ownership status.

A fourth limitation was the imprecision of RAPD-based phylotyping. For example, isolates 28a and 28c, which in the initial RAPD screening were assessed as representing discrete genotypes, were placed as nearest neighbors in the phylotyping dendrogram (Fig. 1) and were found to have identical virulence genotypes (Table 1), evidence that they probably actually represented a single strain. Similarly, the two O18:K1 isolates and the three O6:F48 isolates, which in the initial phylotyping dendrogram were not well resolved (Fig. 1), clearly clustered by serotype in second-round phylotyping (e.g., Fig. 2 and 3). Such imprecision, which in this study occurred despite stringent measures to maximize reproducibility and phylogenetic fidelity, in our experience is an inescapable limitation of amplification fingerprinting (19, 22). This indicates the desirability of a more reliable and reproducible molecular phylotyping method such as multilocus sequence analysis (S. D. Reid, C. Herbelin, A. C. Bumbaugh, R. K. Selander, and T. S. Whittam, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., p. 237).

Bacteremia isolate CP9 was selected as a comparator for canine fecal isolate 12e because of CP9’s status as a model ExPEC strain (39–41, 43–45). In addition, CP9 is genetically indistinguishable from other O4:H5:F13,F14 isolates from women with acute cystitis and from adults with diverse-source bacteremia (28). CP9 was the first E. coli strain other than archetypal pyelonephritis isolate J96 that was found to contain papG alleles I and III (26). papG alleles I and III, together with group III kpsMT, sfa/foc, and cnf, are characteristic of a disseminated J96-like clonal group of E. coli O4:H5, the members of which have caused diverse extraintestinal infections in both humans (26, 28) and animals (unpublished data). This study provides evidence that the J96-like clonal group is present in 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 pap-positive 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 extensively 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 serendipitous discovery of papA sequence homology among these strains. Comparisons of virulence genotypes and RAPD profiles 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-
ical studies to assess transmission rates and associated human health risks.

ACKNOWLEDGMENTS

This material is based upon work supported by Office of Research and Development, Medical Research Service, Department of Veterans Affairs, and National Institutes of Health grant DK-47504 (J.R.J.).

Strains were provided by Gabriele Blum-Oehler (536), Kwang Sik Kim (RS218), Calvin Kunin (US4), Joel Maslow (BOS035), Howard Ochman (ECOR strains), and Ann Stapleton (466). Dave Pretts prepared the figures. Ann Emery helped with manuscript preparation.

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Editor: A. D. O’Brien


