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Feline uropathogenic *Escherichia coli* from Great Britain and New Zealand have dissimilar virulence factor genotypes

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Abstract

We investigated the prevalence of 30 known virulence factor genes (VFGs) in uropathogenic *E. coli* (UPEC) from two geographically distinct feline populations, using a PCR-based approach. *E. coli* isolates were also subjected to macrorestriction analysis to assess their phylogenetic relationships. VFG profiles differed considerably according to the geographic origin of the isolates, enabling discriminant analysis to correctly predict population membership for 15/15 NZ isolates and 18/22 UK isolates. The prevalence of gene markers for P-fimbriae (PapA, PapC, PapEF, and PapG III), colicin V (CvaC), increased serum survival factor (Iss), complement resistance factor (TraT), pathogenicity-associated island (MalX), iron-regulated siderophore receptor (IreA) and haemolysin (HlyD) differed significantly between UK and NZ isolates. Significant phylogenetic differences between the two populations were also identified, but VFG profiles could not be predicted on the basis of phylogenetic relationships. Consequently, a geographically uneven distribution of certain virulence genes, independent of phylogeny, is the likely cause of VFG differences between populations. We cannot rule out that subtle differences in patient disease status may have contributed to the dissimilarity of VFG profiles.

Keywords: Uropathogenic *Escherichia coli*; Virulence factor genotype; Cat; Zoonoses

1. Introduction

Urinary *E. coli* isolates from domestic cats have repeatedly been reported to share similarities with *E. coli* strains that cause serious extraintestinal infections in humans (Yuri et al., 1998; Beutin, 1999; Feria et al., 2001a; Johnson et al., 2001b). In particular, the discovery of a marked species overlap of virulence factor genes (VFGs), elements that enable uropathogenic *E. coli* (UPEC) to persist and multiply within the hosts' urinary tract (Johnson, 2003), has raised concern that humans may acquire UPEC infections from their pet cats (Feria et al., 2001a; Johnson et al., 2001b). Based on phylogenetic data and extended VFG profiles

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from UPEC of very few cats, it has been argued that UPEC from cats and humans may not only share pathogenic traits, but essentially be indistinguishable organisms (Feria et al., 2001a; Johnson et al., 2001b). However, the evaluation of this potential zoonotic risk is not straightforward. One of several factors complicating this field of study is that it is unknown whether different UPEC genotypes are prevalent in different geographic regions. In humans, urinary *E. coli* from different geographic regions have been shown to vary serotypically (Grüneberg and Bettelheim, 1969; Peddie et al., 1981). It is therefore possible that the virulence factor genotypes of urinary *E. coli* infecting any host species also differ with geographic origin. However, while it has been suggested that differences in UPEC isolates related to geographic origin need to be investigated (Johnson et al., 2001b), no study focusing on geographic variation of VFGs within UPEC from one host species has yet been carried out. To contribute to the assessment of the zoonotic risk potential of feline urinary *E. coli*, we applied a previously validated multiplex polymerase chain reaction (PCR) assay (Johnson and Stell, 2000) to determine the prevalence of thirty VFGs of urinary *E. coli* from two geographically distinct feline populations. Furthermore, we investigated the phylogenetic relatedness of these *E. coli* by using macrorestriction analysis. Finally, we examined a possible correlation between PCR-based virulence factor genotype of individual *E. coli* isolates and their phylogenetic profile.

2. Materials and methods

2.1. Strain collection

2.1.1. British strains

Between June 1999 and March 2002, 22 UK strains were isolated from urine samples collected by antepubic cystocentesis from 20 pet cats with suspected urinary tract infection (UTI) and concurrent renal insufficiency or chronic renal failure. Urine samples were collected at two primary care practices in central London where geriatric cat clinics were run by researchers from the Royal Veterinary College, London. The mean age of UK cats was 15 years, ranging from 9 to 20.8. All but one of these patients was female. UK cats were exclusively domestic short haired (DSH).

2.1.2. New Zealand strains

NZ *E. coli* isolates, cultured from urine of exclusively female cats with suspected UTI, were obtained between January 2002 and September 2003 from four animal health laboratories in the North Island of NZ. The mean age in this population was 14 years, ranging from 6 to 18. Five cats were purebred (1 Oriental, 1 Siamese, 3 Burmese), all other cats were DSH.

NZ *E. coli* isolates were derived from urine samples collected by antepubic cystocentesis (12 of 15), or from urine samples for which the collection method was not known, but which showed marked pyuria on sediment evaluation and yielded a pure, heavy growth of *E. coli* (3 of 15).

All isolates were biochemically confirmed as being *E. coli* (Quinn et al., 1994), and stored in 15% glycerol broth at −70 °C until use.

2.2. Virulence factor genotyping

Thirty VFGs were studied using a multiplex PCR as described by Johnson and Stell (2000), with minor modifications. Primer pair combinations are shown in Table 1. Hundred nanograms phenol–chloroform extracted DNA (Ausubel, 1988) template was used per reaction mix. To optimise amplification results 0.5 g/l BSA (New England Biolabs® Inc., Massachusetts, USA) was added to the reaction mixture (Henegariu et al., 1997). Positive amplification of VFGs was identified visually after electrophoresis (1.5% agarose, 0.5 × TBA, 90 V, 1.5 h) and ethidium bromide staining by comparison of band sizes to a 100 bp ladder (Invitrogen™ Life Technologies, Auckland, NZ) and positive *E. coli* control strains L31, J96, V27, PM9, 2H16 (Johnson et al., 1997, 2000, 2001d). Dubious PCR results were clarified by repeating PCR reactions in duplicate using increased or decreased volumes of high quality DNA. DNA integrity was confirmed by amplification of the housekeeping gene marker *aro*E in a separate PCR (Reid et al., 2000).

2.3. Macrorestriction analysis by pulsed field gel electrophoresis (PFGE)

Macrorestriction analysis was done much as described by Alley et al. (2002). The restriction