Uropathogenic *Escherichia coli* of Dogs and Cats:

Pathotypic Traits and Susceptibility to Bacteriophages

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Für meine Eltern

Doris und Dr. rer. nat. Karl-Heinz Freitag

Die ich liebe und respektiere

&

For Derek

Whom I love and respect

The History of Medicine

2006 BC

Here, eat this root.

1000 AD

That root is heathen. Here, say this prayer.

1850 AD

That prayer is superstition. Here, drink this potion.

1920 AD

That potion is snake oil. Here, swallow this pill.

1945 AD

That pill is ineffective. Here, take this penicillin.

1955 AD

Oops . . . bugs mutated. Here, take this tetracycline.

1960–1999 AD

39 more "oops" . . . Here, take this more powerful antibiotic.

2000 AD

The bugs have won! Here, eat this root.

2006 AD

Or maybe try that pill again?

Modified from Anonymous

Abstract

The purpose of this study was to investigate the feasibility of using bacteriophages - viruses that can lyse bacteria - to control infections caused by uropathogenic *Escherichia coli* (UPEC) in dogs and cats. Prior to phage experiments, UPEC were subjected to virulence factor genotyping by multiplex polymerase chain reaction assay and phylogenetic 'fingerprinting' by Pulsed-Field Gel Electrophoresis (PFGE). Twenty-five of 30 assessed virulence factor gene (VFG) markers were detected at least once in 31 UPEC isolated from 20 UK cats and 89 UPEC isolated from dogs (56), cats (22) and people (11) living in New Zealand (NZ). The PFGE banding patterns of UPEC isolates from different individuals were markedly dissimilar unless isolates had been collected at the same hospital within one month of each other. In contrast, ≥2 UPEC strains isolated from each of 3 UK cats diagnosed with multiple UTIs were indistinguishable by PFGE. Antibiograms inaccurately predicted UPEC clonality and, of clinical importance, underestimated the number of relapsing or persistent infections in these cats. A comparison of VFG profiles and PFGE banding patterns of UPEC isolated from NZ and UK cats demonstrated a geographically uneven distribution of pathotypic and phylogenetic traits and indicated that, among other factors, the source of UPEC must be considered when comparing UPEC from different host species. When comparing UPEC isolates from NZ dogs, cats and people, strains with similar VFG profiles were found among the different host species. Other strains, with VFG profiles that differed according to the host species of origin were also detected. The latter finding, which is in contrast to the results of previous studies, may be of interest to researchers aiming to predict the potential zoonotic risk posed by particular UPEC strains sourced from dogs and cats.

Forty bacteriophages (phages for short) were isolated from sewage waters and propagated on UPEC strains. The ability of these phages to cause bacterial lysis was tested on 31 canine UPEC, 22 feline UPEC and 7 faecal *E. coli*. In contrast to faecal *E. coli*, UPEC strains were highly susceptible to phages. Ten phages with a particularly broad host range each lysed ≥27/53 (≥51%) UPEC strains. Used in combination, these 10 phages were predicted to be able to lyse 49/53 (92%) of the UPEC strains in the collection. Morphological and genotypic studies on 5 of these 10 phages demonstrated that 4 of them belonged to the lytic T4-like genus, while one phage showed similarity to the temperate phage P2. Overall, results of this project indicate that the majority of canine and feline UPEC - with very diverse PFGE banding patterns and VFG profiles - are susceptible to lysis by naturally occurring phages. Hence, phages show promise as therapeutic agents for treatment of canine and feline UTI and, perhaps, for other infections caused by UPEC.

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Preface

It has been almost a century since Felix d'Hérelle first applied bacteriophages, viruses that can infect and kill bacteria, to combat bacterial infections in animals and people (reviewed in Summers, 1999). In the first half of last century, bacteriophages (phages for short) were used enthusiastically to treat various bacterial infections (Carlton, 1999; Sulakvelidze et al., 2001; Summers, 2001). However, with the introduction of antibiotics in the early 1940s, interest in phage therapy waned dramatically (Carlton, 1999; Sulakvelidze et al., 2001; Summers, 2001). In recent decades, antimicrobial resistance has become increasingly apparent, generating fear of an impending 'post-antibiotic era' (Alanis, 2005). With the emergence of bacteria that are resistant to multiple antimicrobials, interest in phage therapy has been renewed (Barrow and Soothill, 1997; Merril et al., 2003; Sulakvelidze et al., 2001).

The principal intention of this PhD project was to carry out a preliminary investigation on the feasibility of using phages for treatment or prevention of bacterial infections in dogs and cats. Urinary tract infections (UTIs) caused by uropathogenic *E. coli* (UPEC) were chosen for study. This was because *E. coli* UTI in dogs and cats constitute one of the main infectious disease processes experienced in daily clinical practice (Ling, 2000). *E. coli* UTI can be readily diagnosed by cystocentesis and culture. Thus, it was expected that an adequate number of canine and feline UPEC could be collected in a short time frame. A further reason for focusing on *E. coli* UTI was that these pathogenic *E. coli* have been shown to spread from the urinary tract to other organs (Ling, 2000). There, they may cause serious, intractable infections, such as prostatitis and discospondylitis. These infections and *E. coli* UTI may become increasingly difficult to treat with conventional antimicrobials if the resistance of UPEC to current antimicrobials continues to increase as it has over the last decades (Cohn et al., 2003; Cooke et al., 2002; Mammeri et al., 2005; Sanchez et al., 2002; Warren et al., 2001).

UPEC isolated from dogs and cats were also considered an interesting study, because their role in the pathogenesis of canine and feline UTI is incompletely understood. In particular, few studies have focused on investigating pathotypic traits of feline UPEC in detail (Feria et al., 2000a; Feria et al., 2001a; Feria et al., 2001b; Johnson et al., 2001a; Wilson et al., 1988; Yuri et al., 1998).

In chapter 1, the reader will find a detailed review of the history of phage therapy and current therapeutic applications of phage. Related research areas, such as morphological studies of phage, have been reviewed in brief. The reader will also find a review of the current knowledge concerning canine and feline *E. coli* UTI. Special attention has been given to reporting knowledge of the pathotypic traits studied in detail in this project. This was done because published reviews of pathotypic traits of UPEC do not focus on canine or feline UPEC or do not include a detailed description of all pathotypic traits assessed here (Beutin, 1999; Emody et al., 2003; Hacker and Heesemann, 2000a; Johnson, 1991, 2003; Johnson and Russo, 2005; Mühldorfer et al., 2001).

The materials and methods used to establish the results presented in this thesis are described in detail in chapter 2. The reader will find additional information, such as supplemental information about *E. coli* strains, recipes, primer sequences and suppliers of materials, in the appendix 8.4.

In chapter 3, the first results chapter, pathotypic traits of UPEC that were to become targets of phage were investigated. The work presented in that chapter was seen as an important preparation for the intended *in vitro* phage trials of this project, because a review of the phage therapy literature had shown that (i) lack of knowledge about the targeted bacteria had been associated with phage therapy failure (Carlton, 1999; Summers, 2001); and (ii) a characterisation of pathotypic traits of bacteria may contribute to an understanding of why lysis occurred (Smith and Huggins, 1982). In addition, results presented in chapter 3 complement previous studies that compared canine, feline and human UPEC.

In the early stages of this PhD project, it became apparent that UPEC isolated from New Zealand cats would accumulate more rapidly than anticipated. In addition, the opportunity arose to obtain UPEC from London cats that were concurrently affected by chronic renal failure (CRF). Thus, it was possible to investigate, for the first time, the presence of virulence factor genes (VFGs) in a reasonably large number of feline UPEC isolates. Moreover, by acquisition of feline UPEC from 2 different countries it was possible to assess the possible geographic variation of VFG profiles in feline UPEC. The results of these evaluations are reported in chapter 4.

Among the 31 feline UPEC obtained from the Royal Veterinary College in London were 17 *E. coli* isolates that had been collected from 5 CRF-affected cats that had suffered from multiple UTI during a 2-year period. Much could be learned about recurring UTI in cats from a study of these 17 *E. coli* isolates. Thus, at the time I received these isolates, I was highly enthusiastic about the opportunity to explore the clonal relatedness and antimicrobial resistance patterns of these 17 UPEC isolates. The results of this investigation are reported in chapter 5.

Having characterised the pathotypic traits of "future targets" of phage, it was timely to investigate whether phages able to infect and kill canine and feline UPEC exist in the environment. Chapter 6 describes the results of this investigation and further *in vitro* trials that aimed to determine whether phage therapy could potentially become a useful substitute or supplement to conventional antimicrobial therapy of *E. coli* UTI in dogs and cats.

In chapter 7, the reader will find the general discussion of this thesis. There, important findings of this project have been emphasised and are discussed in detail. Furthermore, strengths and weaknesses of this project have been evaluated. A recommendation for future research that may result out of this project has also been given.

Publications arising from this research are listed in the appendix. Furthermore, the appendices contain useful supplemental information concerning current knowledge of the subject of this thesis, including references to historical publications on phage therapy of UTI. These references are not cited in current search engines and have been very cumbersome to obtain. In addition, the appendices contain raw data concerning experiments described in this thesis and supplemental information to chapter 2 (Materials and Methods).

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ABU	Asymptomatic bacteriuria
CRF	Chronic renal failure
EM	Electron Microscopy
ExPEC	Extraintestinal pathogenic *Escherichia coli*
Fur	Ferric-uptake regulator
HPI	High-pathogenicity island
IVABS	Institute of Veterinary, Animal and Biomedical Sciences
LB	Luria Bertani
MIC	Minimal inhibitory concentration
MLEE	Multilocus enzyme electrophoresis
NCCLS	National Committee for Clinical Laboratory Standards
NZ	New Zealand
OMP	Outer membrane protein
ORF	Open reading frame
PAI	Pathogenicity Island
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
PFU	Plaque forming unit
THP	Tamm-Horsfall protein, synonym uromucoid
UK	United Kingdom
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary tract infection
VF	Virulence factor
VFG	Virulence factor gene