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Genomic, Recombinational and Phylogenetic Characterization of Global Feline Herpesvirus 1 Isolates

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ABSTRACT

Feline herpes virus type 1 (FHV-1) is widely considered to be the leading cause of ocular disease in cats and has been implicated in upper respiratory tract infections. Little, however is known about interstrain phylogenetic relationships, and details of the genomic structure. For the present study, twenty-six FHV-1 isolates from different cats in animal shelters were collected from eight separate locations in the USA, and the genomes sequenced. Genomic characterization of these isolates includied short sequence repeat (SSR) detection, with fewer SSRs detected, compared to herpes simplex viruses type 1 and 2. For phylogenetic and recombination analysis, 27 previously sequenced isolates of FHV-1 were combined with the 26 strains sequenced for the present study. The overall genomic interstrain genetic distance between all available isolates was 0.093%. Phylogenetic analysis identified four main FHV-1 clades primarily corresponding to geographical collection site. Recombination analysis suggested that interclade recombination has occurred.

1. Introduction

Feline herpes virus type 1 (FHV-1) is widely considered to be the leading cause of conjunctival and corneal ulceration in cats (Hartley, 2010) and has been implicated in upper respiratory tract infections as well as a variety of painful ocular conditions including ulcerative keratitis, corneal sequestra, eosinophilic conjunctivitis, uveitis and keratoconjunctivitis sicca (Gaskell et al., 2007). Serological studies indicate that up to 97% of cats have been exposed to the virus (Maggs and Clarke, 2005). More than 80% of cats will become persistently infected following exposure, and 45% will shed virus in response to stressful stimuli (Gaskell and Povey, 1977). The clinical signs of FHV-1 on initial exposure frequently include conjunctivitis, keratitis and upper respiratory disease. This phase is often self-limiting but can result in permanent corneal scarring and symblepharon formation with subsequent blindness (Gould, 2011). Three FHV-1 vaccines are commonly used in the USA, and these are combined with vaccines against feline calicivirus (FCV), and feline panleukopenia virus (FPV) (Reagan et al., 2014). Vaccination against FHV-1 is recommended for all cats, especially young animals in high risk settings, however reduced protection is possible upon intense challenge, and the vaccine will not prevent infection (Thiry et al., 2009).

Although earlier work suggested that FHV-1 was serologically homogeneous (Gaskell and Willoughby, 1999), there have been more recent reports describing differences between isolates identified using PCR techniques. These studies examined the genetic differences between FHV-1 isolates using restriction endonuclease digest to cleave viral DNA (Hamano et al., 2005), and strain differences in viral glycoprotein expression (Hamano et al., 2004). Other papers have evaluated, examined or sequenced smaller individual components of the FHV-1 genome (Hara et al., 1996; Herrmann et al., 1984; Kawaguchi et al., 1994; Maeda et al., 1992; Maeda et al., 1993; Maeda et al., 1995a, b; Willemse et al., 1994). A single strain of FHV-1 was recently fully sequenced (Tai et al., 2010) as well as twenty four clinical isolates from Victoria, Australia and two vaccine strains from the USA (Vaz et al., 2016b). FHV-1 appears to have less intraspecies genomic sequence variability than some other alphaherpesviruses, such HSV-1, HSV-2, SuHV-1, and BHV-1 (Johnston et al., 2017; Kolb et al., 2015;

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Kolb et al., 2017; Newman et al., 2015; Pfaff et al., 2016; Szpara et al., 2014).

FHV-1 is a member of the *Varicellovirus* genus, with an approximately 135,800 bp genome, which is composed of unique long (UL) and unique short (US) sequences, flanked by inverted repeat regions known as terminal and inverted repeat long (TRL, IRL) and inverted and terminal repeat short (IRL, TRS), respectively (Tai et al., 2010). Herpesviruses have been shown to be highly recombinagenic (Loncoman et al., 2017; Norberg et al., 2004; Norberg et al., 2015; Norberg et al., 2007; Razzouk et al., 1996; Schynts et al., 2003; Sijmons et al., 2015; Vaz et al., 2016a), with a slight bias towards the inverted repeat regions (Lee et al., 2015), however *Vaz* et al. (Vaz et al., 2016b) reported that no recombination was detected in FHV-1 in their genomic analysis. It has also been demonstrated that different isolates of FHV-1 have variable virulence in vivo (Gaskell et al., 2007; Hamano et al., 2003) similar to what has been shown in *herpes simplex virus type 1* (HSV-1), *herpes simplex virus type 2* (HSV-2), *equine herpes virus type 1* (EHV-1), and *bovine herpesvirus type 1* (BHV-1) (Brandt and Grau, 1990; Kaashoek et al., 1998; Matsumura et al., 1996; Taha et al., 1989). The role of the host in determining the severity of infection is currently poorly understood, but likely involves aspects of innate and acquired immunity.

The primary objective of the current study was to utilize deep sequencing of the FHV-1 viral genome to more thoroughly evaluate strain variation in shelter-housed cats across the USA and to perform recombination and phylogenetic analysis using global isolate sequence data from previously sequenced isolates of FHV-1 available from Genbank (Tai et al., 2010; Vaz et al., 2016b).

2. Results and Discussion

2.1. Sequencing and genomic assembly

Twenty-six FHV-1 isolates were collected by participating shelters from 8 states across the USA (Fig. 1). The 26 isolates were sequenced using a single lane of the Illumina MiSeq, and the number of sequencing reads for this study ranged from 978,704 (KANS 04) to 1,940,936 (S5727) (Table 1). The quantity of reads mapping to the reference strain following reference assembly ranged from 71,845 (CALI 11) to 775,198 (PHIL 04). The average mapped read length ranged from 272 (KANS 10 and KANS 08) to 281 (MILW 02). The average coverage across the genome ranged from 109× (CALI 11) to 1,023× (PHIL 04).

De novo assembly was also performed on a small subset of the genomes to determine if reference assembly results in a significant increase in genomic variability, which could affect downstream experiments. The results are summarized in Fig. 2A, which shows the differences in single nucleotide polymorphisms (SNPs), insertions/deletions (INDELs), and genome coverage between the CALI 11 reference and *de novo* assemblies. Briefly, the reference and *de novo* assemblies were largely identical, with some small differences. The reference assembly of CALI 11 resulted in complete coverage of the genome, however a small number of SNPs and INDELs were detected in and near the TRL as compared to the *de novo* assembly, and the strain C-27 reference. The CALI 11 *de novo* assembly resulted in reduced coverage of the TRL, and parts of the IRS, TRS, as well as a small region of the US. Five INDELS were also detected near the low coverage area, which we believe are artifacts based on the low coverage. The authors of the study of Australian derived FHV-1 sequences also compared reference to *de novo* assemblies and found them to be identical (Vaz et al., 2016b). The small differences between reference and *de novo* assemblies in this study, specifically the slight SNP asymmetry in the reference assembly, and the small coverage loss from the *de novo* assembly compared to the results by *Vaz* et al. (Vaz et al., 2016b) may be due to CLC-Bio Genomic Workbench (present study) algorithm constraints versus the Geneious package. Because the reference assemblies did not appear to introduce significant amounts of variability, while also resulting in higher coverage, the reference assemblies were used for subsequent analysis. Downstream genomic distance and phylogenetic analysis combining genomes from the current study, as well as previously published genomes (Vaz et al., 2016b) was also deemed unlikely to be significantly affected.

While most of the reference assembled FHV-1 strains exhibited complete coverage, some had reduced coverage in the inverted repeat

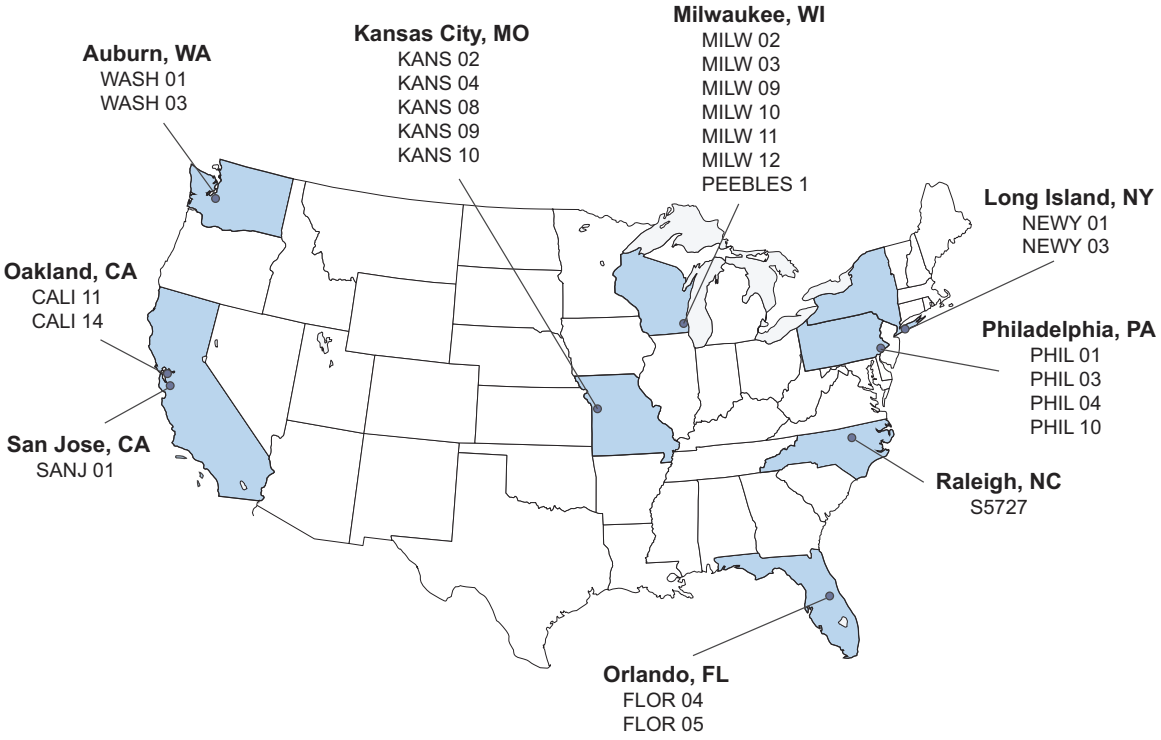

Fig. 1. Sources of viral isolates. The geographic locations and the specific isolates in the USA from which FHV-1 isolates were collected and sequenced are shown.