First Molecular Characterization of Feline Immunodeficiency Virus in Domestic Cats from Mainland China

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Abstract

The feline immunodeficiency virus (FIV) is a retrovirus of the Lentivirus genus that was initially isolated from a colony of domestic cats in California in 1986 and has now been recognized as a common feline pathogen worldwide. To date, there is only one recent serology-based report on FIV in mainland China which was published in 2016. We designed this study to investigate the molecular prevalence and diversity of feline immunodeficiency virus (FIV) in domestic cats from mainland China. We studied the prevalence of FIV in whole blood samples of 615 domestic cats in five cities (Beijing, Guangzhou, Nanjing, Shanghai and Yangzhou) of mainland China and examined them using FRET-PCR (Fluorescence Resonance Energy Transfer-Polymerase Chain Reaction) and regular PCRs for the gag and env genes. Overall, 1.3% (8/615) of the cats were positive for provirus DNA with nucleotide analysis using PCRs for the gag and env sequences showing the cats were infected with FIV subtype A. This is the first molecular characterization of FIV in mainland China and the first description of subtype A in continental Asia.

Introduction

The feline immunodeficiency virus (FIV) is a retrovirus of the Lentivirus genus that was initially isolated from a colony of domestic cats in California in 1986 and has now been recognized as a common feline pathogen worldwide [1–4]. Infected cats may be asymptomatic for many years during which there is progressive disruption of immune function which might lead to a terminal phase with various clinical infections that is referred to as the feline acquired immunodeficiency syndrome [5]. Transmission of FIV is principally by parenteral inoculation of the virus in blood and saliva, presumably during fighting. Male cats are more commonly infected than females and overall prevalence rates in cats vary geographically, mostly from around 2% to 30% [5].
FIV occurs as seven subtypes or clades (A, B, C, D, E, F and U-NZenv) based on nucleotide sequence diversity of the envelope (env) gene [6–9]. The distribution of the clades varies with subtypes A and B being most common, and occurring very widely [8, 10]. Subtype A is common in Australia, New Zealand, the western part of the United States, South Africa and northwestern Europe [8]. Subtype C has been identified in Europe, Africa, Southeast Asia, New Zealand and Canada while subtypes D and E are found only infrequently, originally in Japan, Canada and Argentina [11–14]. Subtype F has only been described from Portugal and the US and the U-NZenv subtype only from New Zealand [7, 9, 15]. There is only limited data on the genotypes of the latter two subtypes.

To date, there have been five FIV-related reports in Taiwan [16–20], but only little data on FIV in mainland China. A study on wild Pallas’ cats from China and other Asian countries identified a unique monophyletic lineage of the FIV most closely related to FIV of African wild cats [21–22].

In the only work on domestic cats, a serosurvey using a commercial test kit (SNAP® Feline Triple® Test, IDEXX Laboratories, Westbrook, ME, USA) found 9% (33/362) of cats studied in Lanzhou, northwestern China, were positive [23]. To provide further information on FIV infections we carried out a molecular survey on cats from five areas in mainland China.

Materials and Methods

The study was reviewed and approved by the Institutional Animal Care and Use Committee of the Yangzhou University College of Veterinary Medicine. Between April 2013 and June 2015, whole blood samples were collected from 615 cats in five cities (Beijing, Guangzhou, Nanjing, Shanghai and Yangzhou) in four provinces of mainland China. The cats from Yangzhou were apparently healthy animals in a shelter while those from the other cities were cats presenting to veterinary clinics for routine health examinations and vaccinations and neutering or with a variety of conditions including fever, stomatitis, and renal failure. All blood samples were collected into EDTA-containing tubes and stored at -80˚C until DNA extraction.

DNA was extracted from whole blood samples with the QIAamp® DNA Blood Mini Kit (QIAGen, Valencia, USA) following the protocol of the manufacturer. A negative control, diethylpyrocarbonate (DEPC)-treated ddH$_2$O, was used for extraction after every 24 blood samples to confirm the absence of carry-over contamination during DNA extraction.

The FIV FRET-PCR was performed in a LightCycler 480-II real-time PCR platform as described previously [24]. This PCR method can detect single copies of a 176-bp gag gene fragment of the FIV provirus genome and can be used to differentiate subtypes A to E [24]. Positive controls consisted of nucleotide fragments of the gag regions of FIV subtypes A, B1, B2/E, C and D that were prepared as described previously [24]. Products obtained in the FIV FRET-PCR were further verified by electrophoresis through 2% agarose gels (BIOWEST®, Hong Kong, China), purified with the QIAquick PCR Purification Kit (Qiagen, Germany), and sequenced with forward and reverse primers (BGI Shanghai, China).

The env sequences of eight FIV subtypes (subtype A: M25381, L00607, X69496, D37813, X69694, M36968; subtype A/B: KP330229; subtype B: D37814, U11820; subtype C: AF474246, AY600517; subtype D: D37811, D37815; subtype E: D84496, D84498; subtype F: DQ072566; subtype U: EF153977, GQ357640) (Fig 1) were obtained from GenBank (www.ncbi.nlm.nih.gov). The Clustal Multiple Alignment Algorithm was used on the V1-V2 and V3-V4 regions common to the env of all the above FIV subtypes to identify polymorphic regions that would enable us to differentiate between subtypes. The primers to amplify the polymorphic regions were synthesized by GenScript (GenScript, Nanjing, China). Standard PCRs were performed with the primers we designed against a 374-bp segment in the V1-V2 region (forward:
Fig 1. Phylogeny of gag and env genes of FIV. Gag sequences (176-bp) of FIV strains identified in this study and representatives of the five subtypes with sequences in GenBank. In addition, a 374-bp region encompassing V1 to V2 is shown on the left of the bottom panel, and a 502-bp region encompassing V3 to V5 on the right panel. The env sequences of the FIV strains identified in this study (in red) are compared with the sequences of representatives of the FIV subtypes with sequences in GenBank; five for V1 to V2 and seven for V3 to V5. Branch lengths are measured in nucleotide substitutions and numbers show branching percentages in bootstrap replicates. Scale bar represents the percent sequence diversity.

doi:10.1371/journal.pone.0169739.g001
GAAGAAGGAAATGCAGGTAAGTTAGAA; reverse: GGTGCCCAACAATCCCAAAA) and a 680-bp segment of V3-V5 (forward: ATACCAAAATGTGGATGGTGAA; reverse: TAATCCTGCTACTGGTATACCAATT). The primers for the V1-V2 region (first segment of the env) amplify subtypes A to E while those for the V3-V5 region (second segment of env) detect all subtypes (A to F and U-NZenv). Positive controls consisted of FIV subtypes A, B and C identified in a previous study [24]. The standard PCRs were performed in a Roche LightCycler II PCR platform. Each reaction was performed with a 20µl final volume containing 10µl of extracted nucleotides, 1×PCR buffer, 1µM forward primer, 1µM reverse primer, 2 unit Taq DNA polymerase and 200µM dNTP. Thermal cycling consisted of 18 high-stringency step-down cycles followed by 30 relaxed-stringency cycles. The cycling parameters for PCR were 6 × 1 sec at 95˚C, 12 sec at 72˚C, 30 sec at 72˚C; 9 × 1 sec at 95˚C, 12 sec at 70˚C, 30 sec at 72˚C; 3 × 1 sec at 95˚C, 12 sec at 68˚C, 30 sec at 72˚C; 30 × 1 sec at 95˚C, 8 sec at 56˚C, 30 sec at 67˚C, 30 sec at 72˚C. Products were verified by gel electrophoresis and sequenced with forward and reverse primers using the Sanger method (BGI, Shanghai, China).

The gag and env sequences we obtained were aligned with similar sequences in GenBank with the Clustalx 1.83 alignment software. Phylogenetic trees were constructed by the neighbor-joining method using the Kimura 2-parameter model with MEGA 6.0. Bootstrap values calculated using 500 replicates.

**Results**

We analyzed blood samples from 615 cats from Beijing (n = 138), Guangzhou (75), Nanjing (146), Shanghai (143) and Yangzhou (113). Background data was available for 514 cats of which 383 were owned and kept mainly indoors and 131 were strays; 278 were male and 236 were female. Estimated age data was available for 458 cats which were placed into one of the following arbitrary age groups: 68 kittens (<6 m), 225 young adults (6 m to 4 yrs), 101 adults (4 to 10yrs) and 64 older cats (>10yrs).

The FRET-PCR followed by confirmatory sequencing showed that 1.3% (8/615) of the cats were positive for FIV. All the FIV-positive cats were male cats from Guangzhou (n = 1), Shanghai (3) and Nanjing (4) (Table 1). Seven of these 8 FIV-positive cats were sick with clinical signs such as stomatitis, salivation and anorexia. The melting point and the gag sequence analyses of the FRET-PCR showed all the positive sequences belonged to FIV subtype A. They had 97%-99% (2-5/164 nucleotide mismatches) similarity with the FIV subtype A TN7 strain (GQ422127) from Canada, and 97%-98% (2-4/164 mismatches) similarity with a FIV subtype A CaONA07 strain (AY225009) from Canada.

The sequences of the V1-V2 env region (GenBank accession number: KX710096- KX710097 and KX904827-KX904832) in the positive cats were all similar (90%-97% identity) with six

| Cat | City     | Age (year) | Gender            | Source                | Health status          |
|-----|----------|------------|-------------------|-----------------------|------------------------|
| C18 | Guangzhou| 1.0        | Neutered male     | Domestic cat          | Renal failure          |
| C180| Nanjing  | 3.0        | Intact male       | Feral cat             | Stomatitis             |
| C181| Nanjing  | 3.0        | Intact male       | Feral cat before adoption | Depression            |
| C171| Nanjing  | 1.5        | Intact male       | Feral cat before adoption | Stomatitis             |
| C172| Nanjing  | 3.0        | Intact male       | Domestic cat          | Stomatitis             |
| C174| Shanghai | 0.25       | Intact male       | Domestic cat          | Fever, 41.3˚C          |
| C176| Shanghai | 3.0        | Neutered male     | Domestic cat          | Feline calicivirus infection |
| C78 | Shanghai | 10.0       | Intact male       | Domestic cat          | Apparently healthy     |

doi:10.1371/journal.pone.0169739.t001
being most closely related to the UK2 strain. This is a FIV subtype A from Scotland (X69494) which has 91% similarity with C18, C172, C176 and C180 (32–34 mismatches) and 93% similarity with C78 and C174 (26 and 28 mismatches, respectively) [25]. In the remaining two positive cats, one (C171) had a strain most closely related to the Sendai1 strain, a FIV subtype A from Japan (D37814) (91% similarity, 32/374), and the other (C181) a strain with 91% similarity to UK2 strain and Sendai1 strain (38/374). (Table 2) [26].

The sequences of the env V3-V5 segment amplicons of the eight positive cats (GenBank accession number: KX646706-KX646707 and KX904833-KX904838) differed by 3%-7% (27–53 mismatches) (Table 3). Five were most closely related to the UK2 strain, a FIV subtype A from Scotland (X69496), with 94–95% similarity (38–43 mismatches) to C171, C172, C174, C176 and C180 (Table 3) [19]. The other three positive strains were most closely related to FIV subtype A/B strain FDSydneyC36 from Australia (KP330229) which had 94% (636/677) identity with C18, 96% (652/683) identity with C78 and 95% (638/680) identity with C181, respectively (Table 3, Fig 1) [26].

The phylogenetic trees we generated (Fig 1) that were based on the nucleotide sequences of our mainland China FIV strains and representative strains of FIV from GenBank clearly demonstrated that our Chinese strains were members of subtype A. In addition, the V3-V5 amino acid sequences of the envelop protein for FIV cats in this study were aligned with those of representative strains of FIV from GenBank (Fig 2).

**Discussion**

The results of our study confirm the presence of the FIV in mainland China and add to the known distribution range of the virus in the country. We found a low prevalence but the cats we studied were predominantly indoor pets that had little contact with other cats. Elsewhere, such cats also have a low prevalence of infection, for example 0.7% in the USA [24]. Why we found no infected cats in the shelter population from Yangzhou is unclear, as feral cats often have a high prevalence of FIV infection, for example 18% in the US [24].

Previous studies have shown cats infected with FIV do not have decreased longevity [27] and that it is only after relatively prolonged infection that immunosuppression occurs and clinical signs become apparent [28]. It was unexpected, then, that seven of the cats we found positive for FIV clinically ill although still relatively young (3 years of age or younger). Unfortunately, there was little or no laboratory data available on these cats and we were not able to establish what, if any, role the FIV infections might have played in the clinical signs that were reported.

Previous studies have shown that PCRs for FIV provirus detection can have a wide range of sensitivities (41–93%) [29]. This relatively poor sensitivity might be as a result of the very low levels of provirus that can be present in infected cats, particularly in apparently healthy animals, but can also be due to variability in the proviral genome of the FIV; there can be up to 26% polymorphism between serotypes in the env and gag [30, 31]. Further, recombination with sometimes complex patterns resulting from co-infections or super-infections is also not uncommon in the FIVs [9, 32]. Because of the wide range of subtypes of FIVs and their high evolutionary rate, it is difficult to develop a PCR that is generic enough to amplify all subtypes and yet maintain high sensitivity [33]. The FRET-PCR we used against the gag has been shown to be sensitive, detecting single copies of the target, and capable of differentiating FIV subtypes A, B, C, D and E [24]. Similarly, the primers we developed against the V1-V2 region of the first segment of the env gene amplified subtypes A to E and enabled their differentiation with sequencing. We could not establish if our primers amplified subtype F and subtype U-NZenv as there are no sequence data for this region on GenBank for these two serotypes. There is
### Table 2. Percent similarities (upper-right diagonal half) and actual numbers of mismatches (lower-left diagonal half) in the env V1-V2 sequences (374bp) of two FIV positive cats from China and representatives of the four FIV subtypes with sequences on GenBank.

|     | C18   | C78   | C171  | C172  | C174  | C176  | C180  | C181  | UK2   | Sendai1 | UK8   | Dixon   | Petaluma | PPR   | FDS   | Sendai2 | USIL   | C     | C36   | Shizuoka | Fukuoka |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|-------|---------|----------|-------|-------|---------|--------|-------|-------|----------|---------|
| C18a| 93    | 90    | 93    | 93    | 90    | 93    | 92    | 91    | 90    | 88      | 90    | 89      | 85       | 76    | 76    | 76      | 76     | 73    | 65    | 65       | 70      |
| C78 | 27    | 94    | 98    | 94    | 94    | 94    | 93    | 92    | 89    | 92      | 92    | 92      | 85       | 76    | 75    | 72      | 72     | 66    | 65    | 71       | 70      |
| C171| 36    | 28    | 93    | 93    | 90    | 94    | 92    | 90    | 91    | 87      | 89    | 88      | 84       | 75    | 73    | 71      | 66     | 66    | 66    | 70       | 70      |
| C172| 28    | 24    | 26    | 94    | 91    | 97    | 94    | 91    | 88    | 91      | 89    | 84      | 77       | 75    | 72    | 66      | 66     | 72    | 71    | 71       | 70      |
| C174| 28    | 7     | 25    | 22    | 94    | 95    | 94    | 93    | 92    | 89      | 92    | 92      | 85       | 76    | 75    | 72      | 66     | 65    | 71    | 70       | 70      |
| C176| 38    | 22    | 40    | 34    | 22    | 91    | 91    | 91    | 90    | 88      | 90    | 89      | 84       | 77    | 76    | 73      | 66     | 65    | 71    | 69       | 70      |
| C180| 28    | 22    | 24    | 12    | 19    | 34    | 95    | 91    | 88    | 91      | 90    | 84      | 76       | 74    | 71    | 66      | 66     | 66    | 72    | 71       | 70      |
| C181| 31    | 28    | 32    | 24    | 26    | 38    | 21    | 91    | 91    | 87      | 90    | 89      | 84       | 75    | 74    | 72      | 67     | 66    | 71    | 70       | 70      |
| A-UK2| 33   | 28    | 37    | 32    | 26    | 34    | 32    | 38    | 40    | 38      | 38    | 38      | 38       | 38    | 38    | 38      | 38     | 38    | 38    | 38       | 38      |
| A-Sendai1 | 38 | 29    | 32   | 34   | 28    | 38    | 34    | 38    | 37    | 38      | 88    | 91      | 84       | 76    | 74    | 72      | 66     | 66    | 66    | 69       | 69      |
| A-UK8 | 43   | 41    | 37    | 43    | 40    | 46    | 46    | 53    | 44    | 44      | 88    | 87      | 90       | 76    | 75    | 72      | 66     | 66    | 66    | 69       | 69      |
| A-Dixon | 38  | 31    | 43    | 35    | 31    | 37    | 34    | 41    | 34    | 36      | 44    | 91      | 84       | 76    | 74    | 72      | 64     | 65    | 70    | 70       | 67      |
| A-Petaluma | 42 | 31    | 46    | 40    | 31    | 43    | 38    | 46    | 37    | 37      | 49    | 34      | 85       | 77    | 76    | 74      | 66     | 66    | 72    | 70       | 70      |
| A-PPR | 56   | 55    | 62    | 61    | 56    | 61    | 59    | 63    | 65    | 60      | 60    | 57      | 76       | 75    | 72    | 66      | 65     | 69    | 67    | 67       | 67      |
| A/B-FDS | 91  | 91    | 95    | 89    | 90    | 87    | 93    | 98    | 96    | 92      | 90    | 89      | 91       | 95    | 94    | 68      | 68     | 68    | 70    | 71       | 71      |
| B-Sendai2 | 94 | 97    | 102   | 96    | 96    | 93    | 101   | 104   | 100   | 99      | 96    | 94      | 96       | 19    | 97    | 67      | 67     | 67    | 70    | 70       | 70      |
| B-USIL2489 | 103 | 106   | 113   | 107   | 107   | 104   | 111   | 112   | 109   | 108     | 107   | 102     | 105      | 12    | 66    | 65      | 68     | 68    | 69    | 68       | 69      |
| C-C | 133   | 131   | 131   | 129   | 133   | 133   | 130   | 130   | 133   | 129     | 131   | 136     | 128       | 131   | 132   | 129     | 134    | 95    | 63    | 64       |         |
| C-C36 | 133  | 134   | 132   | 130   | 136   | 136   | 130   | 133   | 137   | 132     | 131   | 140     | 130       | 134   | 124   | 130     | 137    | 20    | 64    | 65       |         |
| D-Shizuoka | 114 | 111   | 114   | 107   | 111   | 113   | 107   | 115   | 114   | 105     | 115   | 105     | 120       | 111   | 114   | 119     | 143    | 92    |       |         |         |
| D-Fukuoka | 116 | 114   | 116   | 110   | 116   | 117   | 110   | 118   | 119   | 125     | 125   | 115     | 125       | 110   | 113   | 118     | 140    | 139   | 32    |         |         |

*The GenBank Accession numbers of the China strains are C18 (KX710096); C78 (KX710097), C171 (KX904827), C172 (KX904828), C174 (KX904829), C176 (KX904830), C180 (KX904831) and C181 (KX904832), while those of previously reported FIV are: subtype A, UK2 (X69494), Sendai1 (D37813), UK8 (X69496), Dixon (L00607), Petaluma (M25381), PPR (M36988); subtype A/B, FDSydney C36 (KP330229); subtype B, Sendai2 (D37814), USIL2489 (U11820); subtype C, C (AF474246), C36 (AY600517); subtype D, Shizuoka (D37811), Fukuoka (D37815).*
Table 3. Percent similarities (upper-right diagonal half) and actual numbers of mismatches (lower-left diagonal half) in the env V3-V5 sequences (C18:677bp and C78:683bp) of two FIV positive cats from China and representatives of each of the seven FIV subtypes with sequences on GenBank.

|          | C18 | C78 | C171 | C172 | C174 | C176 | C180 | UK8  | Sendai1 | Sendai2 | USIL | C   | C36 | Shizuoka | Fukuoka | LP3 | LP20 |
|----------|-----|-----|------|------|------|------|------|------|---------|---------|------|-----|-----|----------|---------|-----|------|
| C18^a    | 94  | 93  | 94   | 93   | 94   | 94   | 94   | 94   | 94      | 94      | 94   | 94  | 94  | 95       | 94      | 94  | 94   |
| C78      | 95  | 96  | 95   | 95   | 96   | 95   | 95   | 95   | 95      | 95      | 95   | 95  | 95  | 97       | 95      | 95  | 95   |
| C171     | 53  | 53  | 54   | 54   | 53   | 53   | 53   | 53   | 53      | 53      | 53   | 53  | 53  | 53       | 53      | 53  | 53   |
| C172     | 44  | 44  | 44   | 44   | 44   | 44   | 44   | 44   | 44      | 44      | 44   | 44  | 44  | 44       | 44      | 44  | 44   |
| C174     | 53  | 54  | 55   | 55   | 54   | 54   | 54   | 54   | 54      | 54      | 54   | 54  | 54  | 54       | 54      | 54  | 54   |
| C176     | 51  | 51  | 51   | 51   | 51   | 51   | 51   | 51   | 51      | 51      | 51   | 51  | 51  | 51       | 51      | 51  | 51   |
| C180     | 51  | 51  | 51   | 51   | 51   | 51   | 51   | 51   | 51      | 51      | 51   | 51  | 51  | 51       | 51      | 51  | 51   |
| C181     | 48  | 48  | 48   | 48   | 48   | 48   | 48   | 48   | 48      | 48      | 48   | 48  | 48  | 48       | 48      | 48  | 48   |
| UK8      | 56  | 56  | 56   | 56   | 56   | 56   | 56   | 56   | 56      | 56      | 56   | 56  | 56  | 56       | 56      | 56  | 56   |
| A-Sendai1| 50  | 50  | 50   | 50   | 50   | 50   | 50   | 50   | 50      | 50      | 50   | 50  | 50  | 50       | 50      | 50  | 50   |
| A-UK2    | 56  | 55  | 55   | 55   | 55   | 55   | 55   | 55   | 55      | 55      | 55   | 55  | 55  | 55       | 55      | 55  | 55   |
| A-Dixon  | 66  | 66  | 66   | 66   | 66   | 66   | 66   | 66   | 66      | 66      | 66   | 66  | 66  | 66       | 66      | 66  | 66   |
| A-PPR    | 68  | 68  | 68   | 68   | 68   | 68   | 68   | 68   | 68      | 68      | 68   | 68  | 68  | 68       | 68      | 68  | 68   |
| A-Petaluma| 73  | 73  | 73   | 73   | 73   | 73   | 73   | 73   | 73      | 73      | 73   | 73  | 73  | 73       | 73      | 73  | 73   |
| A/B-FDS  | 41  | 41  | 41   | 41   | 41   | 41   | 41   | 41   | 41      | 41      | 41   | 41  | 41  | 41       | 41      | 41  | 41   |
| B-Sendai2| 143 | 143 | 143  | 143  | 143  | 143  | 143  | 143  | 143     | 143     | 143  | 143 | 143 | 143      | 143     | 143 | 143  |
| B-USIL2489| 145 | 145 | 145  | 145  | 145  | 145  | 145  | 145  | 145     | 145     | 145  | 145 | 145 | 145      | 145     | 145 | 145  |
| C-C      | 156 | 156 | 156  | 156  | 156  | 156  | 156  | 156  | 156     | 156     | 156  | 156 | 156 | 156      | 156     | 156 | 156  |
| C-C36    | 153 | 153 | 153  | 153  | 153  | 153  | 153  | 153  | 153     | 153     | 153  | 153 | 153 | 153      | 153     | 153 | 153  |
| D-Shizuoka| 160 | 160 | 160  | 160  | 160  | 160  | 160  | 160  | 160     | 160     | 160  | 160 | 160 | 160      | 160     | 160 | 160  |
| D-Fukuoka| 144 | 144 | 144  | 144  | 144  | 144  | 144  | 144  | 144     | 144     | 144  | 144 | 144 | 144      | 144     | 144 | 144  |
| E-LP3    | 138 | 138 | 138  | 138  | 138  | 138  | 138  | 138  | 138     | 138     | 138  | 138 | 138 | 138      | 138     | 138 | 138  |
| E-LP20  | 136 | 136 | 136  | 136  | 136  | 136  | 136  | 136  | 136     | 136     | 136  | 136 | 136 | 136      | 136     | 136 | 136  |

The GenBank Accession numbers of the China strains are C18 (KX646706), C78 (KX646707), C171 (KX904833), C172 (KX904834), C174 (KX904835), C176 (KX904836), C180 (KX904837) and C181 (KX904838), while those of previously reported FIV are: subtype A, UK8 (X69496), Sendai1 (D37813), UK2 (X69494), Dixon (L00607), PPR (M36968), Petaluma (M25381); subtype A/B, FD Sydney C36 (KP330229); subtype B, Sendai2 (D37814), USIL2489 (U11820); subtype C, C (AF474246), C36 (AY600517); subtype D, Shizuoka (D37811), Fukuoka (D37815); subtype E, LP3 (D84496), LP20 (D84498).

doi:10.1371/journal.pone.0169739.t003
sequence data, however, for the V3-V5 region of all the FIV subtypes and the primers we developed for this second segment of the env were capable of detecting all subtypes, that is A to F and also U-NZenv. The PCRs we performed in our study thus enabled us to detect low copy numbers of FIV and also to detect all the recognized subtypes.

In our study, all the FIV positive isolates we detected belonged to subtype A which occurs widely around the world with most isolates being from Australia, New Zealand, North America, South Africa and Europe [10, 34]. Isolates from countries closer to mainland China have included subtypes A, B, C and D from Japan [14], subtype C from Korea and Vietnam [13, 35], and subtype D from Thailand [36]. Our description of subtype A in mainland China is thus the first description of this subtype in the country and, to the best of our knowledge, on the mainland of Asia.

Of note is our finding that the sequences of the second segment of the env in three of our mainland China FIV strains (C18, C78 and C181) were very similar to the FDSydneyC36 (41, 31 and 42 mismatches, respectively) (Table 3, Fig 2). The sequences of the first segment of the env, however, were relatively distant (91, 91 and 98 mismatches, respectively), being more distant (91, 91 and 98 mismatches, respectively).
closely aligned with representatives of the FIV subtype B (Table 2, Fig 2). This difference is explained by the fact that the FDSydneyC36 strain, from a cat immunized with a commercial FIV vaccine [26], is a recombinant strain of FIV, subtype A/B. The second segment of the env is assigned to subtype A while the first segment is assigned to subtype B.

Our findings of FIV subtype A and the serological evidence of infections presented by Cong et al. [23] should alert Chinese veterinarians to the possibility of infections in their feline patients. Although clinical signs resulting from FIV infection are highly variable and unpredictable, cats infected with subtype A have been found to remain asymptomatic for longer and have lower viral loads than cats infected with subtype C [34, 37, 38]. The subtype A FIV strains are often neurotrophic and can produce neurological signs, most commonly behavioral changes but also seizures, paresis, multifocal motor abnormalities, impaired learning and disrupted sleep patterns [5]. Currently there is only one registered FIV vaccine which is composed of two FIV subtypes, A and D. The vaccine is reported to confer protection against subtypes A, B and D and might then be useful in mainland China where [32, 39], to the best of our knowledge, vaccination is seldom if ever performed. A recent study, however, has shown the vaccine does not confer solid protection and breakthroughs were found with FIV subtypes A, F, A/F and D/F [40]. Further studies on the usefulness of vaccination under conditions of natural challenge are required, particularly in Asian countries where subtype C is prevalent.

In conclusion, our study has shown that FIV subtype A occurs in mainland China and continental Asia. Larger studies are indicated to further determine the subtypes present in the region which will facilitate the development of accurate diagnostic tools and control programs.

Acknowledgments

This project was supported by grant from the National Natural Science Foundation of China (NO 31272575, 31472225), and the Priority Academic Program Development of Jiangsu Higher Education Institutions and China Scholarship Council.

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Funding acquisition: CW.
Investigation: JL LW JZ.
Methodology: CW PK.
Project administration: CW.
Resources: JZ CW.
Software: JZ SP CW.
Supervision: CW PK.
Validation: PK SP.
Visualization: CW JL JZ.
Writing – original draft: CW PK JZ.
Writing – review & editing: CW PK JZ JL SP.
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