PAPER

European molecular epidemiology and strain diversity of feline calicivirus

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Context

Feline calicivirus (FCV) causes a variable syndrome of upper respiratory tract disease, mouth ulcers and lameness. It is associated with lymphoplasmacytic gingivitis stomatitis complex (LGSC) and as an RNA virus, FCV evolves quickly and is extremely diverse. This is seen in phylogenetic analyses of genomic sequence data, which typically result in a 'star-like' phylogeny with many strains cocirculating. This FCV strain diversity is a major challenge for vaccine cross-protection, with vaccine antigens chosen to be broadly cross-reactive. Attempts to identify robust groupings of FCV either on temporal, clinical or spatial grounds have largely failed. This is in contrast to the related calicivirus causing gastroenteritis in people (human norovirus), where a major strain variant circulates internationally. However, there have been no attempts to analyse the international diversity of FCV. This study aims to describe the diversity of FCV in a population of cats attending veterinary practices chosen by convenience in five European countries.

Main conclusion

The strain diversity of FCV was confirmed in five European countries. Although strains persist and circulate locally there was no evidence for widescale national or international dispersal of individual strains.

Approach

A convenience-based prospective cohort of cats was recruited by veterinary surgeons in 13 sites in five countries in Europe (five sites in France, all in the Paris area), two in the UK (Bangor in Northern Ireland and Northampton), two in Portugal (Mem Martins and Lisbon area), one in Greece (Thessaloniki), and three sites in Germany (Lippstadt, Berlin and Achim). Individual cats were recruited either through attending the veterinary clinic, or when a veterinary surgeon from the clinic visited a local cat shelter.

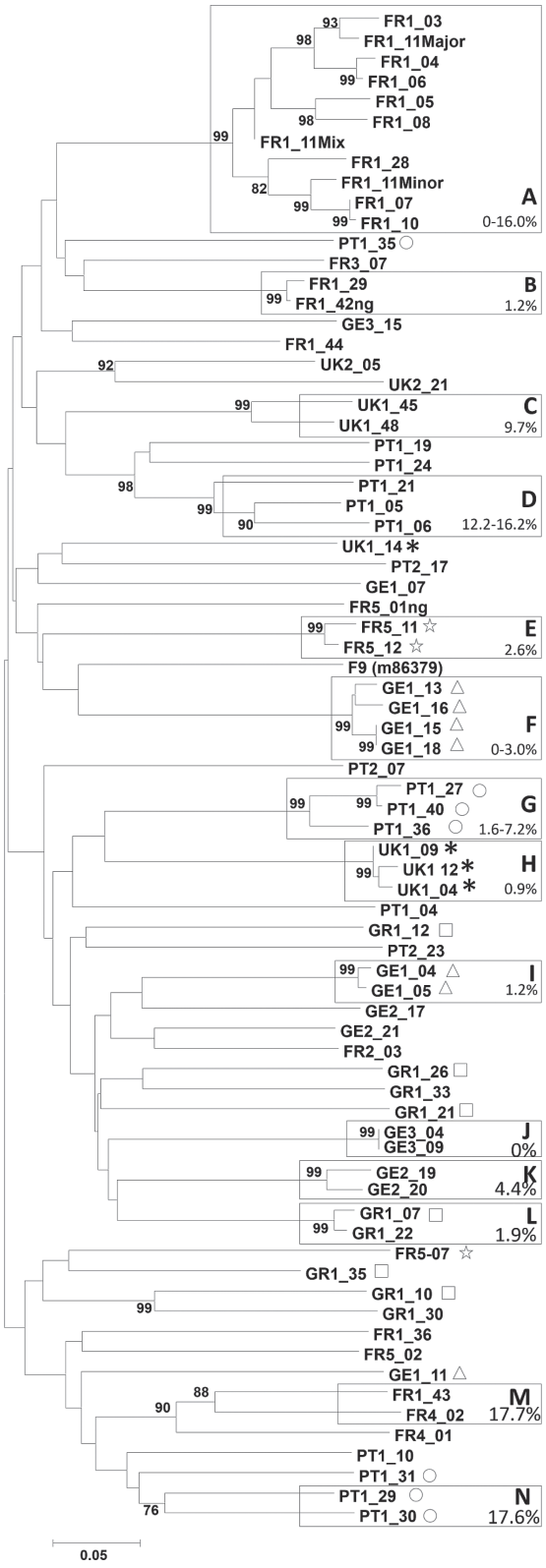

FIG 1: Unrooted Kimura 2-parameter Neighbour joining tree of partial capsid sequences from this study and FCV vaccine strain F9. Each sequence has a unique ID made up of a country code (FRance, GErmany GReece, PorTugal and United Kingdom) and site number (1 to 5)_ sample number. Strains represented by more than a single sequence (less than 20 per cent divergence) are boxed, additionally labelled A-N and the intra-strain capsid diversity indicated in the box. Where multiple sequences came from a single household they are indicated by an additional symbol (□, o, Δ, *, ☆). The percentage of replicate trees in which the associated taxa clustered together in bootstrap tests (1000 replicates) is shown next to the branches (only bootstrap values greater than 75 per cent are shown). Distances are drawn to scale and relate to the distance bar.

From each cat, an oropharyngeal swab and a questionnaire completed by the owner were collected. Diagnosis of FCV and feline herpesvirus type 1 (FeHV-1) was determined by virus isolation. Risk factors potentially associated with FCV infection were assessed by multivariable analyses. Sequence and phylogenetic analyses of capsid and polymerase sequences were used to analyse strain diversity, evolution and transmission between countries.

Results

A total of 426 samples were collected. For 17 samples, the viral status could not be assessed due to bacterial overgrowth. For the remaining 409, FCV and FeHV-1 were isolated from 91 (22.2 per cent) and 18 (4.4 per cent) cats, respectively. For FCV, 16.2 per cent of healthy cats and 34.2 per cent of sick (at least one clinical sign) cats tested positive. For FeHV-1, the figures were 2.6 and 8.0 per cent. Multivariable analysis found that animals presenting with LGSC were 9.33 (95 per cent confidence interval 3.18 to 29.45) times more likely to present with FCV infection than cats without LGSC. Furthermore, vaccinated cats up to 48 months old were significantly less likely to be infected with FCV than unvaccinated animals of similar ages.

Phylogenetic analysis based on consensus sequences for the immunodominant region of the capsid gene from 72 FCV isolates identified 46 strains (pairwise genetic distance greater than 20 per cent) (fig1). There was no evidence of widescale clustering at the geographical level; rather strains from each country were dispersed throughout the tree. Thirteen of the 14 strains with more than one sequence were restricted to individual regions or sites in individual countries with the exception being a strain present in two sites close to each other in France. Four strains were present in more than one household. Five colonies (four of which were rescue shelters) had multiple strains within them. Polymerase sequences suggested possible rare recombination events.

Interpretation

The study confirms the close association between FCV and LGSC, and provides evidence for a protective effect of FCV vaccination against infection in young cats. Phylogenetic analyses point to a range of transmission dynamics and reaffirm the role of multicat households and shelters as potential generators of strain diversity. These locally, nationally and internationally diverse FCV populations maintain a continuous challenge to the control of FCV infection and disease. This was a 'convenience' sample of cats and FCV isolates, so may not be completely representative of the general population.

Significance of findings

High levels of strain diversity point to the continued rapid evolution of FCV. A lack of dominant circulating strains reaffirms the need to choose vaccine antigens on their ability to be broadly cross-reactive.