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Research paper

Pattern of seroreactivity against feline foamy virus proteins in domestic cats from Germany

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ABSTRACT

The prevalence of feline foamy virus (FFV, spumaretrovirinae) in naturally infected domestic cats ranges between 30 and 80% FFV positive animals depending on age, sex and geographical region analyzed. Two serotypes have been reported for FFV designated FUV7-like and F17/951-like. Serotype-specific neutralization has been shown to correlate with sequence divergence in the surface (SU) domain of the envelope protein (Env). We analyzed a serum collection of 262 domestic cat sera from Germany using a GST-capture ELISA setup screening for Gag and Bet specific antibodies and identified 39% FFV positive animals. Due to the heterogeneity of the serological samples, cut-offs for Gag and Bet reactivity had to be experimentally determined since application of calculated cut-off values yielded some false-positive results; the new cut-off values turned out to be also fully applicable to a previous study. Using the already established FUV7 ElpSU antigen and the newly cloned and produced F17/951 ElpSU antigen, both consisting of the corresponding ectodomains of the envelope leader protein (Elp) and SU protein, we aimed at the detection of Env-specific antibodies and discrimination between the two known FFV serotypes within the diagnostic FFV ELISA. We validated the ElpSU antigens using cat reference sera of known serotype and screened with this assay domestic cat sera from Germany. Use of the FUV7- and F17/951 ElpSU antigens in ELISA resulted in the detection of Env-specific antibodies in both cat reference sera and sera from domestic cats in Germany, but failed to allow serotyping at the same time.

1. Introduction

Foamy viruses (FVs), also known as spumaretroviruses, are a distinct subfamily within the *Retroviridae* with distinguishing features in their replication pathway and a complex genomic organisation (Yu et al., 1996; Neumann-Haefelin et al., 1993; Linial, 1999; Bastone et al., 2003; Rethwilm, 2003). FV infections are persistent and infected animals show a sustained antibody response against Gag and Bet that is used for serological identification of infected hosts via ELISA and/or immunoblotting (Alke et al., 2000; Hahn et al., 1994; Heneine et al., 2003; Khan and Kumar, 2006; Saib, 2003; Williams and Khan, 2010). Virus can commonly be isolated from infected cats, cattle and non-human primates (Alke et al., 2000; Heneine et al., 2003; Khan and Kumar, 2006; Romen et al., 2007; Saib, 2003; Williams and Khan, 2010); however, no disease was associ-

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ated with infections and thus, FVs are therefore considered apathogenic (Linial, 2000; Saib, 2003). In addition, zoonotic infections of human beings by simian FVs have been described but are not associated with an overt disease (Heneine et al., 2003; Khan, 2009).

Based on serum neutralization assays and later confirmed by molecular sequencing, 11 different serogroups have been recognized among simian FVs (SFV; Hooks and Gibbs, 1975; McClure et al., 1994; Bieniasz et al., 1995; Schweizer and Neumann-Haefelin, 1995). Interestingly, more than one SFV serotype has been found to infect one species of primates and co-infection of a single animal with different SFVs has been reported (Hooks et al., 1972; Leendertz et al., 2008). In cats, two distinct serotypes of FFV have been identified due to differential neutralization patterns (Hackett and Manning, 1971; Mochizuki and Konishi, 1979; Flower et al., 1985). FFV serotypes 951, F17 and others (Riggs et al., 1969; Helps and Harbour, 1997) represent the F17/951-like serotype, whereas the FUV7-like viruses comprise the second serotype. Sequence comparison of the two FFV serotypes revealed a very high overall amino acid sequence homology of the Gag, Pol, Bel1/Tas and Bet proteins whereas Env displayed high conservation of Elp and the N-terminal part of SU plus the whole TM (Winkler et al., 1998). In contrast, the C-terminal end of SU displayed only 57% amino acid identity between the two serotypes whereas within each serotype, homology of this region was again high, with 97% identity (Winkler et al., 1998). This sequence divergence in Env SU, limited to a defined region, has been recently shown to correlate with serotype-specific neutralization of FFV isolates (Zemba et al., 2000). In contrast to SFVs, super-infection by different serotypes does not seem to be the case for FFV, but only a small number of cats have been analyzed and such rare events may have therefore been overlooked (Winkler et al., 1998). Currently, no data is available for bovine and equine foamy viruses concerning different serotypes.

One goal of this study was to determine the prevalence of FFV in German domestic cats using field sera that had been collected and provided by practicing veterinarians and also to determine with these sera the robustness of our recently established ELISA-based FFV screening system. In addition, we studied whether utilization of the divergent Env sequences of the two FFV serotypes FUV7 and F17/951 allows serotyping by GST-ELISA technology. This would open the possibility of detecting within one assay not only Gag-, Bet- and Env-specific antibodies but also of determining the FFV serotype.

2. Materials and methods

2.1. Molecular cloning and recombinant proteins

The ectodomain of the Elp-SU part of FFV serotype F17/951 was amplified by PCR with primers FUV-F17 ElpSU-s (5′-CGTATCGAATTCTCAATGGAAAGAAGCAATAACAC-3′) and FUV-F17 ElpSU-as (5′-AGCAGTGTCGACTTGTCTTCTACCTTTCTTTCTTTC-3′) introducing restriction sites for *Eco*RI and *Sal*I, respectively, and plasmid pczFFVenv as template (Picard-Maureau et al., 2003). PCR was done using hot start high fidelity DNA polymerase (Roche, Mannheim, Germany) at 94 °C for 2 min plus 30 cycles of 94 °C for 30 s, 54 °C for 1 min and 72 °C for 2 min. The PCR product was digested with *Eco*RI and *Sal*I cleaving at the introduced sites, purified by gel electrophoresis and cloned into the correspondingly treated pGEX4T3tag derivative (Sehr et al., 2002). The F17/951 Elp-SU ectodomain was fused in frame between the 5′ GST domain and the 3′ SV40-tag (KPPTPPPEPET). Clones were identified by restriction enzyme digestion and DNA sequencing.

For fusion protein expression, *E. coli* BL21 or *E. coli* BL21 Rosetta cells were transformed with pGEX-X-tag plasmids and recombinant proteins (Gag, Bet, FUV-7 ElpSU, F17/951 ElpSU) were purified as described (Sehr et al., 2001, 2002).

2.2. Cat sera used in this study

German domestic cat sera were sampled in veterinary practices under unknown conditions and sent to a diagnostic laboratory under standard postal conditions; aliquots of 262 cat sera of unknown FFV immune status were kindly provided by Dr. Janine Hübner and Susanne Kolb (LABOKLIN, Bad Kissingen, Germany).

Cat reference sera 10, 12, 14, 24, and 26 were collected from homeless cats that had previously been pets in different households in Adelaide, South Australia in 1996/1997. The FFV serotype of the respective viruses was determined as published (Winkler et al., 1998). As positive control in ELISA and immunoblots, the serum from an experimentally FFV FUV-infected female cat (cat 8014) was used (Alke et al., 2000).

Sera from 24 specific pathogen free (SPF) domestic cats were used to determine background reactivity of sera drawn, stored and shipped under optimal conditions. Twelve samples were obtained from the Colorado State University SPF cat breeding colony and an additional 12 samples originated from SPF cats housed at CSU following purchase from a commercial SPF USDA Class A vendor. Blood was collected via cephalic vein on conscious animals following protocols approved by the CSU Institutional Animal Care and Use Committee. Serum was collected following centrifugation and stored at −80 °C until submitted for FFV serologic analysis.

2.3. GST capture ELISA and cut-off definition

ELISAs were performed as previously described (Sehr et al., 2001, 2002; Romen et al., 2006). 96-well titer plates were coated with glutathione casein, pre-adsorbed with blocking buffer (0.2% (w/v) casein in PBS, 0.05% (v/v) Tween-20) and then 100 μl cleared *E. coli* lysates containing the GST-tag or GST-X-tag fusion proteins (0.25 μg total protein in blocking buffer) were added.

Cat sera were pre-incubated in blocking buffer containing 2 μg/μl total protein from GST-tag-expressing *E. coli* BL21 at a dilution of 1:50. Pre-adsorbed sera were incubated for 1 h at RT in the coated plates, washed and incubated for 1 h at RT with Protein A–peroxidase conjugate (Sigma–Aldrich, Germany). Substrate reaction and quantification were done as described (Sehr et al., 2001). All incubations were performed with a volume of 100 μl per well.