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Molecular characterization and virus neutralization patterns of severe, non-epizootic forms of feline calicivirus infections resembling virulent systemic disease in cats in Switzerland and in Liechtenstein

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ABSTRACT

Feline calicivirus (FCV) infections are associated with oral ulceration, chronic stomatitis and a limping syndrome. Epizootic outbreaks of virulent systemic disease (VSD) have been reported in the USA and Europe. Here, the molecular characterization and neutralization patterns of FCV isolates from cases of severe, non-epizootic infection associated with skin ulceration and edema are presented. Samples from eleven symptomatic cats, four in-contact cats and 27 cats with no contact with symptomatic cats were collected and tested for FCV, feline herpesvirus-1 (FHV-1), feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV). Phylogenetic analyses based on the capsid (VP1) gene of FCV and virus neutralization with antisera raised against four FCV vaccine strains were performed. Nine kittens and two adult cats in two shelters and two veterinary clinics in four geographically distinct locations in Switzerland and Liechtenstein were affected. The cats showed fever, tongue and skin ulceration, head and paw edema, and occasionally jaundice, generalized edema and dyspnea. All symptomatic cats tested FCV-positive but were negative for FHV-1, FeLV and FIV, with the exception of one FIV-positive kitten. All kittens of one litter and both adult cats died. The disease did not spread to cats in the environment. Cats in the environment displayed phylogenetically distinct, but related, FCV strains. Virus neutralization patterns suggested that some cases might have been potentially prevented by vaccination with the optimal vaccine strain. In conclusion, clinicians should be aware of severe, non-epizootic forms of FCV infections with initial clinical presentations similar to VSD.

1. Introduction

Feline calicivirus (FCV) is a highly infectious RNA virus of the family *Caliciviridae* and one of the most common viral pathogens in cats worldwide (Radford et al., 2009). The virus is detected in up to 40% of cats living in large groups (i.e. colonies or shelters) and in about 10% of privately owned cats living alone or in small groups (Bannasch and Foley, 2005; Coutts et al., 1994; Helps et al., 2005; Radford et al., 2001; Wardley et al., 1974). FCV exhibits a remarkably high genetic evolution rate, which is thought to result

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from genetic drift or recombination (Coyne et al., 2006a; Coyne et al., 2007; Coyne et al., 2006c). Consequently, genetically diverse FCV isolates can be isolated among naturally infected cats (Coyne et al., 2012). It has been postulated that such genetic variation might favor the persistence of FCV in groups of cats, leading to the emergence of novel strains (Coyne et al., 2006a; Coyne et al., 2007; Coyne et al., 2006c).

Typical of vesivirus infections, FCV has been associated with vesicular disease (Pesavento et al., 2008). Acute infections are characterized by transient fever and ulcerations on the tongue and palate of affected cats (Radford et al., 2009). In more severe cases, oral fauces, gingiva, lips and nasal philtrum may also be ulcerated. Another clinical presentation of FCV infection is the limping syndrome associated with transient lameness and acute synovitis (Radford et al., 2009). FCV has also been assigned to the feline upper respiratory tract disease (URTD) complex; however, classical signs of URTD in FCV-infected cats are often caused in conjunction with other viral or bacterial pathogens (Binns et al., 2000; Cai et al., 2002; Helps et al., 2005), and not all FCV isolates induce respiratory disease following experimental challenge (Pesavento et al., 2008). FCV is also present in a high proportion of cats displaying chronic lympho-plasmacytic gingivitis/stomatitis (Radford et al., 2009). This syndrome has so far not been successfully reproduced by experimental FCV infection (Knowles et al., 1991; Poulet et al., 2000) and is thought to represent an immune-mediated disease (Harley et al., 1999). In its most severe clinical form, FCV infection induces a highly contagious virulent systemic disease (VSD), which is characterized by a systemic inflammatory response syndrome (Pedersen et al., 2000). The disease involves internal organs as well as skin and mucous membranes. Affected cats show edema and skin ulceration, mainly around the head and limbs, and occasionally jaundice, dyspnea and bleeding tendencies (Coyne et al., 2006b; Pedersen et al., 2000; Radford et al., 2009; Schorr-Evans et al., 2003; Schulz et al., 2011). Epizootic outbreaks of VSD were first reported in cats in North America, but subsequently also in Europe (Coyne et al., 2006b; Hurley et al., 2004; Pedersen et al., 2000; Reynolds et al., 2009; Schorr-Evans et al., 2003; Schulz et al., 2011). The outbreaks usually occur in multi-cat environments and have been characterized by rapid onset and spread and high mortality (Radford et al., 2009). Published data suggest that these highly virulent strains emerge independently from genetically distinct FCV strains (Coyne et al., 2006b; Ossiboff and Parker, 2007; Reynolds et al., 2009; Schulz et al., 2011), but attempts to identify genetic patterns within the viral genome that define the highly virulent FCV biotype have been inconclusive (Abd-Eldaim et al., 2005; Foley et al., 2006; Prikhodko et al., 2014; Rong et al., 2006). Controversial results have been published concerning the protective effect of FCV vaccination against VSD. Most naturally infected cats developed VSD despite regular vaccination (Hurley et al., 2004; Schorr-Evans et al., 2003). However, experimental infection with a virulent-systemic FCV isolate resulted in a milder, self-limiting course in cats vaccinated with the FCV vaccine strain F9 when compared to unvaccinated cats (Pedersen et al., 2000).

In 1972, Cooper and Sabine described a cat with paw edema, oral lesions and skin ulcerations and called the syndrome 'paw and mouth disease' (Cooper and Sabine, 1972); FCV was isolated from tongue and paw lesions of the affected cat. The initial clinical presentation of this syndrome was similar to that reported as VSD, but the syndrome lacked high mortality, obvious organ involvement and epizootic disease spread. In the present case series, we report eleven cases of severe, non-epizootic forms of FCV infections associated with ulcerative lesions on the head and limbs and cutaneous edema that occurred in four unrelated geographic locations in Switzerland and Liechtenstein. The study describes clinical data from the cases and presents the molecular characterization and analysis of susceptibility to neutralization of the FCV isolates from the affected cats.

2. Material and methods

2.1. Case definition, sample and data collection

Cases were included when they met the following criteria: (1) ulcerative lesions on the head and limbs and/or the presence of

Table 1
Results for FCV, FHV-1, FeLV and FIV of symptomatic cats and of healthy in-contact cats. Positive results are shown in bold.

Location	Cat	Samples collected[a,b]	Date of sampling	FCV RT-qPCR	FHV-1 PCR[f]	FeLV[f]	FIV[f]
Shelter 1	Case 1	OC, blood	Nov 2011	**Positive**[c]	Negative	Negative[g]	Negative[i]
	Case 2	OC, blood	Nov 2011	**Positive**[c]	Negative	Negative[g]	Negative[i]
	Case 3	OC, blood	Nov 2011	**Positive**[c]	Negative	Negative[g]	Negative[i]
	Case 4	OC, blood	Nov 2011	**Positive**[c]	Negative	Negative[g]	Negative[i]
	Case 5	OC, blood	Nov 2011	**Positive**[c]	Negative	Negative[g]	Negative[i]
	Queen	OC	Nov 2011	**Positive**	nt	nt	nt
	In-contact cat 1	OC	Nov 2011	**Positive**	nt	nt	nt
	In-contact cat 2	OC	Nov 2011	**Positive**	nt	nt	nt
	In-contact cat 3	OC	Nov 2011	**Positive**	nt	nt	nt
Clinic 1	Case 6	OC, blood	Jul 2012	**Positive**[c,d]	Negative	nt	nt
		Edema and pustule fluid	Jul 2012	**Positive**	nt	nt	nt
Shelter 2	Case 7	OC, blood	Aug 2012	**Positive**[c]	nt	Negative[g]	Negative[i]
		OC/NS/CS	Oct 2012	**Positive**	Negative	Negative[h]	Negative[j]
	Case 8	OC/NS/CS	Oct 2012	**Positive**	Negative	Negative[h]	Negative[j]
Shelter 2	Case 9	OC/NS/CS	Jan 2013	**Positive**	Negative	Negative[h]	Negative[j]
	Case 10	OC/NS/CS	Jan 2013	**Positive**	Negative	Negative[h]	**Positive**[j]
Clinic 2	Case 11	OC/NS/CS, blood	April 2014	**Positive**[d,e]	Negative	Negative[g]	Negative[i]
		Mucosa, skin and liver	May 2014	**Positive**	nt	nt	nt

[a] OC, oropharyngeal cytobrush.
[b] OC/NS/CS, pooled material from oropharyngeal cytobrush, nasal and conjunctival swabs.
[c] FCV RT-qPCR positive in the OC.
[d] FCV RT-qPCR positive in blood.
[e] FCV RT-qPCR positive in the OC/NS/CS.
[f] nt, Not tested.
[g] Result of FeLV ELISA from blood.
[h] Result of FeLV RT-qPCR from OC/NS/CS.
[i] Result of FIV ELISA from blood.
[j] Result of FIV RT-qPCR from OC/NS/CS (for details see Section 2).