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Short communication

First report of *Feline Calicivirus* (FCV) infection in stray cats in northeast China

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Abstract

To improve our understanding of *Feline calicivirus* (FCV) infection in cats in Northeast China, 1584 serum samples from 974 domestic cats and 610 stray cats were collected between 2012 and 2015. The samples were tested for FCV antibodies using a commercially available ELISA kit. The results revealed an overall seroprevalence of 37.56% (595/1584), a seroprevalence in domestic cats of 32.85% (320/974) and a seroprevalence in stray cats of 45.08% (275/610). Risk factor analysis indicated that species was the only risk factor for the presence of FCV (OR=1.678, 95% CI=1.362-2.066, $P<0.001$); age, season, region and gender were not risk factors. This is the first report of FCV infection in stray cats in China, and the results of this study can aid in FCV infection control in the felidae family.

Key words: *Feline calicivirus*, seroprevalence, ELISA

Introduction

Feline calicivirus (FCV) is a small, unenveloped, single-stranded RNA virus of the *Vesivirus* genus of the calicivirus family. Both domestic cats and other members of the felidae family can be infected with FCV (Munson et al. 2004, Driciru et al. 2006, Harrison et al. 2007). Studies have also demonstrated FCV infection in dogs (Martella et al. 2002), which suggests a potential risk for cross-species transmission. FCV strains differ in tropism and virulence; therefore, infection presents with a wide range of clinical signs. Most strains induce a fairly characteristic, mild syndrome characterized by pyrexia, oral ulceration, and mild respiratory and conjunctival signs. However, some FCV strains are nonpathogenic, and others are more virulent than the most common strains, with the ability to induce more severe systemic disease. Several outbreaks of a severe acute systemic febrile disease with high mortality have been reported (Martella et al. 2002, Di Martino et al. 2009); most outbreaks occurred in the United States and Europe, and they were caused by particularly virulent strains of FCV (FCV-associated virulent systemic disease, FCV-VSD). The impact of this disease is not limited to cats, and its ability to infect wildcats should not be ignored.

Materials and Methods

Northeast China (40°-53° N, 120°-135° E), including Liaoning province, Jilin province and Heilongjiang province, has a total area of 80.84 square kilometres.

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Table 1. Seroprevalence of FCV infection in cats, Northeast China, by enzyme-linked immunosorbent assay (ELISA).

Factor	Category	No. tested	No. positive	Prevalence (%)
Age (years)	0 < years ≤ 1	541	210	38.82
	1 < years ≤ 2	199	63	31.66
	2 < years ≤ 4	339	97	28.61
	4 < years	505	170	33.66
Season	spring	467	195	41.76
	summer	354	124	35.03
	autumn	335	106	31.64
	winter	428	170	39.72
Gender	male	471	182	37.84
	female	1113	413	37.11
Species	domestic cats	974	320	32.85
	alley cats	610	275	45.08
Region	jilin	974	337	34.59
	liaoning	227	93	40.96
	heilongjiang	383	165	43.08
Total		1584	595	37.56

The climate is characterized by a cold, long winter and warm, short summer. Serum samples were randomly collected from domestic and stray cats in different areas of Northeast China between 2012 and 2015. Detailed information, including age, gender, species and region of the cats as well as season at the time of collection were recorded and are listed in Table 1. The serum samples were tested for antibodies against FCV using a commercially available ELISA kit (DRG, New Jersey, USA) according to the manufacturer's instructions (Javier and Alejandro, 2009). Positive and negative controls were provided with the kit.

Seropositive cats were analysed in terms of age, gender, species and region and season at the time of sample collection. An exploratory analysis was performed to identify variables associated with exposure to FCV infection. Potential risk factors were studied using a multivariable logistic regression model; a probability (p) value < 0.05 was considered to indicate statistically significant differences in FCV antibody levels between cats with different risk factors. Odds-ratios (OR) with 95% confidence intervals based on likelihood ratio statistics are reported. All statistical analyses were performed using the PASW Statistics 19.0 software (SPSS Inc., IBM Corporation, Somers, NY).

Results and Discussion

FCV causes multiple oral and upper respiratory diseases in domestic cats and wildcats and is epidemic and highly infectious. It is clinically referred to as feline rhinoconjunctivitis. FCV not only harms domestic cats and wildcats but also seriously threatens other members of the felidae family, such as tigers. Protection of these large animals of the felidae family is critical. Although antigenicity is not generally associated with pathogenicity, some evidence suggests that chronic stomatitis isolates have minor antigenic differences (Dawson et al. 1993, Poulet et al. 2000). In this study, the overall FCV seroprevalence in cats was 37.56% (595/1584) (Table 1), which is lower than that reported in wildcats (80%) in Spain (Javier et al. 2009), but higher than that reported in cats in California, USA (13%-36%) (Bannasch et al. 2005) and in rescue shelter cats in South Wirral, UK (31%) (Radford et al. 2001). Many factors may contribute to such differences, such as geographical and climatic conditions, species, breeding method, and sensitivity of the detection technique.

The FCV seroprevalences in cats of different age groups ranged from 33.22% to 40.31%, but this difference was not statistically significant (p>0.05). The highest seroprevalence was observed in the ≤ 1-year-old age group (40.31%). This result is similar to that of a recent study (Helps, et al. 2005), and may be explained by the fact that the immune systems of cats ≤ 1 year-old may not be as robust as those of older cats or by the fact that kittens typically endure numerous stressful events, such as vaccination, rehoming, and neutering.

Some studies have shown prevalences of approximately 10% in areas with few domestic cats and prevalences up to 25%-40% in areas with dense cat populations (Radford et al. 2001, Bannasch and Foley 2005, Helps et al. 2005). The average prevalence reported in individual cats ranges from 6% to

Table 2. Odds ratios for specie as risk factor for FCV seroprevalence in cats (n = 1584).

Factor	Group	Prevalence(%)	OR	95%CI	*P*-value
Species	domestic cats	32.85	reference		
	alley cats	45.08	1.678	1.362-2.066	<0.001

75% (Coyne et al. 2006). FCV mainly infects kittens aged 6-84 days, and it is commonly found in kittens aged 56-84 days; mortality is higher in infected kittens of decreasing age in days. In this study, the seroprevalence in kittens was higher than that in older cats, which is similar to previous reports.

FCV is a member of the genus calicivirus of Caliciviridae, and it is most commonly infects members of the cat family. In cats, FCV infection can induce aphthous ulcers, rhinoconjunctivitis, other symptoms and even death (Schorr-Evans et al. 2003, Coyne et al. 2006, Reynolds et al. 2009, Battilani et al. 2013). Once a cat is infected with FCV, it remains infected for life. However, because the FCVs are relatively short-lived outside of cats, the environment is not usually a long-term source of infection. Therefore, FCV prevalence in different seasons should not be related to infection risk. In this study, FCV seroprevalence varied between seasons from 31.64% to 41.76% (Table 1), but this difference was not statistically significant (p>0.05). The possible cause of the high seroprevalence in winter and spring is that FCV can survive longer in colder wetter conditions.

FCV seroprevalences in different regions ranged from 34.59% to 43.08%. In general, the seroprevalence in Jilin province was lower than that in Heilongjiang province; however, this difference was not statistically significant (p>0.05). These differences may be due to the antigenic diversity of FCV and the inability of current vaccines to provide equal protection against all FCV strains.

The results of this study showed that cat species was the only risk factor associated with FCV seroprevalence. The logistic regression analysis showed that stray cats (OR = 1.678, 95% CI = 1.362-2.066, p<0.001) had a higher risk of FCV exposure than domestic cats (Table 2); there are multiple possible explanations for this difference. First, in China, although most domestic cats are vaccinated, this study revealed a relatively low antibody level (32.85%) (Table 1), indicating that the protective effect of the current vaccine is insufficient. This may be due to high variation in FCV strains, particularly different virulent strains. Second, most stray cats are unvaccinated, and the detection rate of FCV antibodies in stray cats was 45.08% (Table 1); this indicates that the majority of stray cats are infected with FCV and that they are the main source of FCV infection and viral transmission. Third, domestic cats live in areas of low cat density with generally stable cat populations. In contrast, stray cats do not have permanent homes; therefore, they are exposed to variable cat populations.

FCVs are mainly shed in ocular, nasal, and oral secretions and are mainly spread by direct contact with an infected cat. Many infected cats are fully vaccinated, suggesting that the currently available vaccines do not completely protect against virulent systemic disease-producing isolates. Although past outbreaks have been relatively well-controlled by strict quarantine and isolation, given that FCV is an inherently variable pathogen, clinicians must remain vigilant with future outbreaks of this disease. This is the first report of FCV seroprevalence in cats in China. Because of the presence of vaccine-induced antibodies, the true natural infection history of FCV, especially in domestic cats, could not be determined by the ELISA assay used in this study. However, the antibody level in domestic cats was only 32.85% (Table 1), therefore, the effect of vaccine-induced antibodies was limited. The aim of this study was to determine the risk factors associated with FCV infection in 1584 by measuring seroprevalence; FCVs and FCV RNA were not directly studied.

Limited information is available regarding FCV infection in cats, especially stray cats, in northeast China. This study not only adds to the available epidemiological data but also has significance for determining methods to protect rare wild felidae animals. This study provides a theoretical basis for developing prevention and control measures for FCV infection, which is becoming increasingly severe. Stray cats may carry and transmit FCV despite being asymptomatic; this generates great risk for the development of sustainable infection in other cats and felines. Therefore, we should improve our monitoring of animal FCV epidemics.

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