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ISOLATION AND IDENTIFICATION OF FELINE CALICIVIRUS AND FELINE HERPESVIRUS IN SOUTHERN BRAZIL

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ABSTRACT

Feline calicivirus (FCV) and feline herpesvirus type 1 (FHV-1) are the two primary causes of upper respiratory tract disease in cats. The aim of this study was to demonstrate the distribution of FCV and FHV-1 among the feline population of several counties in Rio Grande do Sul State, Brazil. To this end, conjunctival and nasal swabs were collected from 302 cats from different locations, including households, breeding catteries, veterinary clinics, animal hospitals and experimental research facilities. The samples were collected between July 2006 to June 2009. The virus isolation was performed in CRFK cells and, subsequently, the identification was confirmed by PCR. FCV, FHV-1, or both were isolated from 55 cats from 28 different locations. FCV alone was isolated from 52.7% (29/55) of the animals that tested positively, FHV-1 alone was isolated from 38.2% (21/55) of the animals that tested positively, and co-infection were detected in 9.1% (5/55) of the animals that tested positively. Virus detection was more prevalent in cats that were less than 1 year old, among animals that shared a living space with other cats, and females. FCV and FHV-1 were isolated from vaccinated cats. In addition, both viruses were isolated from cats that showed no signs of disease. The results suggest that a carrier state is common for both viruses in the evaluated population. A search for other causes of respiratory disease in that population is necessary; and further studies relating to the molecular characterization of viruses and vaccine efficacy are also necessary.

Key words: FCV, FHV-1, URTD, epidemiology

INTRODUCTION

Infectious respiratory disease is a major clinical problem in feline medicine. Such infections are primarily caused by either one or both of two viruses: feline calicivirus (FCV) and feline herpesvirus type 1 (FHV-1) (11, 25). These viruses have

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worldwide distribution, and it is estimated that roughly 80 percent of upper respiratory tract disease (URTD) infections in cats are caused by FCV and FHV-1 (12, 17).

FCV is a virus that belongs to the *Vesivirus* genus and the *Caliciviridae* family. It has a small, single-stranded, positive-sense RNA genome that encodes three open reading frames [ORFs] (25). Individual FCV isolates may differ, but they all belong to a single serotype (25). Clinical disease caused by FCV is typically characterized by oral ulcerations with or without mild respiratory and conjunctival signs (25). The virus has also been associated with the presence of transient and shifting lameness, hemorrhagic-like fever (21), abortion (5) and chronic stomatitis (16). Following recovery from acute disease, cats may become a carrier, effectively shedding the virus from the oropharynx (24, 31). The duration of this carrier state is variable and ranges from months to years in individual animals (9). Higher prevalence of FCV has been associated with cats of less than 12 months and households were large number of cats are housed together (2, 3, 30).

More recently, and more worryingly, highly virulent strains of FCV have emerged that are associated with outbreaks of disease with high mortality and a new range of clinical features, FCV- associated virulent disease (VSD) - previously haemorrhagic-like fever (21, 25). In addition to upper respiratory tract disease, the affected cats show to varying degrees pyrexia, cutaneous oedema, ulcerative dermatitis, anorexia and jaundice, with up 50 per cent of them dying or being euthanased in extremis (4). Adult cats are frequently affected more severely than kittens, and vaccination does not appear to be protective (4). FCV can be isolated from oral and conjunctival swabs from affected cats (4). VSD-FCV has not yet been described in Brazil.

FHV-1 is the agent of feline viral rhinotracheitis (FVR) (11, 8). It is a DNA virus that is a member of the *Varicellovirus* genus in the *Alphaherpesvirinae* subfamily (11). Only one serotype of this virus exists, and like other *alphaherpesvirus*, FHV-1 induces latency in nervous ganglions (11). Thus, clinically recovered cats are carriers that undergo periodic episodes of virus reactivation, particularly after stress (11). In both experimental and natural infections, symptoms include the following: depression, sneezing, inappetence, pyrexia and serous ocular and nasal discharges (11). Cats of any age, sex or breed are susceptible, but a severe syndrome is usually restricted to kittens of up to six months of age (23). It is estimated that more than 90% of the cats are seropositive to FHV; and that a minimum of eighty percent remains latently infected with 45% shedding the virus by all life long (18).

The nasal, oral and conjunctival vias are the natural routes of infection for FCV and FHV-1 (11, 25). Transmission occurs mainly through direct contact between infected and susceptible cats; however, indirect transmission can also occur in the case of FCV, particularly within a cattery where secretions may contaminate cages, feeding and cleaning utensils or personnel (11, 25). Reports from cats with URTD have revealed a prevalence ranging among 20-53% for FCV and 10-34% for FHV-1 (2, 12, 16). In the general healthy cat population from several European countries, USA and Korea, the prevalence of FCV has varied from 15% to 31% (2, 16) whereas values ranging from less than 1% to 63% have been estimated for the prevalence of FHV-1 (2, 13, 15).

These viruses are still prevalent in the feline population despite the fact that vaccination against FCV and FHV-1 has been practiced since the 1970s (2, 29). Vaccinations may have reduced the overall severity of disease; however, in some vaccinated individuals, disease may still occur (11, 25, 29). Commercially available vaccines are generally safe and protect reasonably well against disease, although they do not prevent infection, the shedding of virus or even the development of the carrier state (9, 11, 20, 25). Live attenuated and inactivated vaccines are available (11, 25), and vaccine virus shedding after vaccination has been described experimentally only for a temperature sensitive FHV-1 vaccine (33). In that case, vaccine virus has been shed for 25 days after vaccination (33).

There is little available information about FCV and FHV-

1 in Brazil. Vaccination is performed in Veterinary Clinics and Hospitals with live attenuated and inactivated vaccines however the percentage of the population that is actually vaccinated is unknown. The isolation of FCV has been described once in the southern part of the country (32), followed by an experimental study regarding the pathogenicity of the virus (22). However, to our knowledge, there has not yet been a described isolation of FHV-1 in Brazil. Regardless, evidence of the presence of both viruses has been obtained from serologic surveys performed in populations of wild (6, 27) and domestic felines (14). The aim of the present study was to generate insights into the epidemiology of FCV and FHV-1 in the southern part of Brazil. Thus, conjunctival, nasal, oral and oropharyngeal swabs were collected from 302 cats in several counties in Rio Grande do Sul State, and the resulting isolation and identification of FCV and FHV-1 are described.

MATERIALS AND METHODS

Source of samples

The samples consisted of conjunctival, nasal and, occasionally, oral and oropharyngeal swabs collected from cats with or without clinical signs of respiratory disease. The samples were collected from July 2006 to June 2009 from 302 cats from veterinary clinics and hospitals, residences, breeding catteries and experimental populations. The samples were collected from animals in the following counties of the Rio Grande do Sul State, Brazil: Cachoeira do Sul, Canoas, Estrela, Nova Palma, Pelotas, Porto Alegre, Santa Maria and Santo Ângelo. All of the animal handling procedures were performed under veterinary supervision and following the recommendations of the Brazilian Committee on Animal Experimentation (COBEA, law #6.638 of May, 8th, 1979). The experiments were approved by an institutional committee on animal welfare and ethics (UFSM - approval number # 61/2009).

Cell culture and virus isolation

The feline kidney cell line CRFK (*Crandell-Rees feline kidney*) was used for virus isolation and amplification. Cells were routinely maintained in Eagle's minimal essential medium (MEM) containing penicillin (1.6 mg/L), streptomycin (0.4 mg/L), amphotericin B (2.0 mg/L), and 10% fetal calf serum.

Swabs were kept in microtubes with MEM medium (0.5 ml) and stored at – 70°C until use in experiments. The swabs were briefly agitated in a vortex and the content was then transferred to microcentrifuge tubes and centrifuged at 10.000 x *g* for 5 min. The supernatants (0.15 ml) were inoculated onto CRFK cell monolayers grown in 24-well plates and were submitted to three passages of five days each while the cells were monitored for cytophatic effect (CPE). Cultures exhibiting CPE were investigated for the presence of feline calicivirus (FCV), feline herpesvirus type 1 (FHV-1), or both viruses using a polymerase chain reaction (PCR) assay. Three blind passages were performed for cultures not exhibiting CPE, and the cultures were considered negative for virus isolation.

Extraction of DNA and RNA, primers and PCR

Only one sample from each animal was tested by PCR and RT-PCR, including those from which the viruses were isolated from different swabs. Different samples isolated from the same cat were pooled to perform the PCR/ RT-PCR. The extraction of FCV RNA and FHV-1 DNA was performed using TRIzol and DNAzol reagents (Invitrogen, Carlsbad, CA, USA), respectively. The extractions were performed from cell cultures inoculated with the respective viruses following the manufacturer's protocol. After extraction, DNA was solubilized in 8 mM NaOH (0.2 ml) and stored at – 20°C until further testing. The RNA was solubilized in 30 µl of ultra-pure water with DEPC (diethylpyrocarbonate) and stored at -70°C until use. The cDNA was synthesized in 20 µl of total solution containing the following: 2 µl of RNA (approximately 100 ng), 100 ng of random primers, 10X buffer from reverse transcriptase (RT), 25 mM $MgCl_2$, 10 mM dNTPs, 0.1 Mm DTT, 40 U RNaseOUT and 200 U RT (SuperScript^{TM} III RT – Invitrogen). The solution was incubated at 65°C for 5 min,

25°C for 10 min, 42°C for 50 min and 85°C for 5 min. The cDNA was used as a template for the PCR for FCV identification.

The ORF2 (regions B to F), which encodes the major capsid protein (25), was the target region for amplification of the FCV cDNA. The amplified product resulted in a fragment of 955 bp (base pairs). The primer sequences were 8F (forward) 5' – CACSTTATGTCYGACACTGA – 3' (position 6142 B region) and 8R (reverse) 5' – CTRGADGTRTGCA RRATTT – 3' (position 7097 F region), based on the FCV-F9 strain (GenBank access number M86379). The primers used were degenerate. The letters S, Y, R and D refer to C/G, T/C, A/G, and A/C/T, respectively, and were previously developed (19). The PCR conditions used were as it follows: 94°C for 5 min for the initial denaturation, followed by 35 cycles of three steps of 94°C for 45 s, 48°C for 45 s, 72°C for 45 s, and a final extension of 7 min at 72ºC.

For FHV-1 the thymidine kinase enzyme (TK) gene was the region of the viral DNA amplified by PCR. The primer set used in the reaction was previously developed (28), and the product size is 287 bp (GenBank access number M26660). The primer sequences used were as follows: Herp_F (forward) 5' – GACGTGGTGAATTATCAGC – 3' (position 510 TK gene) and Herp_R (reverse) 5'– CAACTAGATTTCCACCAGGA – 3' (position 797 TK gene). The PCR conditions used were as follows: 94°C for 5 min, followed by 40 cycles at 94°C for 45 s, 56°C for 30 s, 72°C for 45 s and a final extension of 7 min at 72°C.

The resulting PCR products were electrophoresed in 1.5% agarose gel, stained with ethidium bromide and visualized under UV light. A commercial live attenuated vaccine to both viruses plus *Chlamydophila felis*, Felocell CVR-C (Pfizer Animal Health, USA), was used as a control in standardizing the PCR reactions and as a positive control in all of the tests. Two of the samples isolated in our lab and identified by electron microscopy (EM), SV65/90 (FCV) and SV534/00 (FHV-1); were also used as positive control in the reactions. Negative controls consisted of mock-infected CRFK cells.

RESULTS

A total of 572 swabs samples were collected from 302 domestic felines either with or without clinical signs suggesting infection by feline calicivirus (FCV) and/or feline herpesvirus type 1 (FHV-1). Most of the samples were conjunctival or nasal swabs; only in two cases and one case were the swabs collected from the oral and oropharyngeal cavities, respectively (Table 1). The feline population sampled in this article consisted of household cats living alone, household cats living with other cats, breeding cattery cats, hospital cats, cats in veterinary clinics and cats kept isolated for experimental research. The data were organized according to animal age, gender, vaccination status, habitat, presence or absence of clinical signs and the type of swabs collected (Table 1).

Table 1. General description of the feline population and the distribution of virus isolation among the samples collected from domestic cats from July 2006 to June 2009.

Epidemiologic aspect	Overall (%)	Virus isolation (%)	
		Positive	Negative
Total of cats	302	55 (18.2)	247 (81.8)
Age (years)			
< 1 year	116 (38.4)	24 (20.7)	92 (79.3)
1 - 5 years	144 (47.7)	27 (18.8)	117 (81.2)
5 - 10 years	34 (11.2)	4 (11.8)	30 (88.2)
> 10 years	8 (2.6)	0 (0)	8 (100)
Gender			
female	146 (48.3)	37 (25.3)	109 (74.7)
male	142 (47)	14 (9.9)	128 (90.1)
not informed	14 (4.6)	4 (28.6)	10 (71.4)
Vaccination status			
vaccinated	89 (29.5)	14 (15.7)	75 (84.3)
not vaccinated	186 (61.6)	36 (19.3)	150 (80.7)
not informed	27 (8.9)	5 (18.5)	22 (81.5)
Habitat			
single	27 (8.9)	2 (7.4)	25 (92.6)
with other cats	253 (83.7)	52 (20.6)	201 (79.4)
not informed	22 (7.2)	1 (4.5)	21 (95.5)
Clinical signs			
presence	70 (23.2)	25 (35.7)	45 (64.3)
absence	232 (76.8)	30 (12.9)	202 (87.1)
Swabs collected	572	73 (12.8)	499 (87.2)
conjunctival	289 (50.5)	32 (11.1)	257 (88.9)
nasal	280 (49)	38 (13.6)	242 (86.4)
oral	2 (0.3)	2 (100)	0 (0)
oropharyngeal	1 (0.2)	1 (100)	0 (0)

Virus detection and identification

FCV, FHV-1, or both were detected in 55 from the 302 cats examined in this survey (Tables 1 and 2). FCV alone was isolated in 52.7% (29/55) of the cats that tested positively, FHV-1 alone in 38.2% (21/55) and double infection was detected in 9.1% (5/55) (Table 2). Virus isolation was confirmed in all cases by PCR and RT-PCR for FHV-1 and FCV, respectively.

Table 2. Characteristics of the 55 positive cats in terms of isolation of feline calicivirus (FCV), feline herpesvirus type 1 (FHV-1) or both viruses.

Epidemiologic aspect	Virus isolation (%)			
	FCV	FHV-1	Both	Overall
Total	29 (52.7)	21 (38.2)	5 (9.1)	55
Age (years)				
< 1 year	11 (45.8)	9 (37.5)	4 (16.7)	24 (43.6)
1 - 5 years	16 (59.3)	10 (37)	1 (3.7)	27 (49.1)
5 - 10 years	2 (50)	2 (50)	- (0)	4 (7.3)
Gender				
female	21 (56.8)	14 (37.8)	2 (5.4)	37 (67.3)
male	8 (57.1)	6 (42.9)	- (0)	14 (25.4)
not informed	- (0)	1 (25)	3 (75)	4 (7.3)
Vaccination status				
vaccinated	3 (21.4)	10 (71.4)	1 (7.1)	14 (25.4)
not vaccinated	23 (63.9)	10 (27.8)	3 (8.3)	36 (65.4)
not informed	3 (60)	1 (20)	1 (20)	5 (9.1)
Habitat				
single	2 (100)	- (0)	- (0)	2 (3.6)
with other cats	27 (51.9)	21 (40.4)	4 (7.7)	52 (94.5)
not informed	- (0)	- (0)	1 (100)	1 (1.8)
Clinical signs				
presence	11 (44)	13 (52)	1 (4)	25 (45.5)
absence	18 (60)	8 (26.7)	4 (13.3)	30 (54.5)

Epidemiological and clinical aspects

Virus was isolated from cats showing clinical signs of disease and from healthy cats (Table 1). The opposite also held true, as virus was not detected in samples from cats with evident clinical manifestations of respiratory disease (Table 1). The clinical signs most frequently observed in the 25 cats with evidence of disease were as follows: ocular discharge in 16/25 (64%); nasal discharge in 7/25 (28%); conjunctivitis, sneezing, coughing, dyspnea, fever and anorexia in 6/25 (24%); and oral lesions (ulcer) in 3/25 (12%). Respiratory disease was observed in 24 out of the 25 cats that tested positively for virus isolation, and 2 of them also showed oral ulcers. Only 1 of the 25 cats only showed oral ulcers without other signs of respiratory disease. FCV alone was isolated from the 3 cases in which ulcers were detected.

FCV and FHV-1 were isolated from both vaccinated and non-vaccinated cats. When each situation is considered separately, the results reveal that 15.7% (14/89) of the total population of vaccinated cats tested positively for one or both viruses, whereas approximately 19% (36/186) of the non-vaccinated cats tested positively. A comparison of the data relating vaccine status to the presence or absence of clinical signs did not reveal a substantial difference among the vaccinated and non-vaccinated groups. The analysis demonstrated that 40% of the 25 cats that exhibited clinical signs and from which virus was isolated were vaccinated, whereas 48% were not vaccinated. In addition, among the 30 cats that tested positively but did not show signs of disease,

approximately 43% were not vaccinated and 13.3% were vaccinated (data not shown).

The 55 cats that tested positively for viral isolation came from 28 different locations. FCV alone was isolated from 17 (60.7%), FHV-1 alone from 7 (25%), and both viruses in four out of the 28 places (14.3%) [data not shown]. FCV alone was isolated from eight of thirteen cats kept together for experimental research. The cats from the breeding catteries were found to be only positive for FHV-1. There were six positive cats in each of two catteries and two cats from another breeding cattery. Among the cats from the veterinary hospitals, which came from four different locations, FCV was detected in two locations; both viruses were detected in the samples coming from cats obtained from these two locations. FCV was isolated from 66.6% (12/18), FHV-1 from 22% (4/18) and both viruses were isolated from 11.1% (2/18) of the samples coming from the remaining eighteen locations sheltering cats living with another cat or cats (data not shown).

With regards to age, approximately 93% of the 55 cats exhibiting positive results for virus isolation were between 0 and 5 years old. Taking into consideration cats from which only one of the viruses or both viruses where isolated, FCV was isolated more often from cats between 1 and 5 years old, whereas FHV-1 was isolated more often from cats under 1 year old (Table 2).

The classification by gender revealed that the difference between the number of samples collected from male and female cats was only two, although the number of females that tested positively for virus isolation was more than two-fold higher the number of males that tested positively (Table 2). With regards to the origin of the cats, females were positive in 16 locations, males in 9 locations and both male and female in 3 locations (data not shown).

DISCUSSION

The epidemiological conditions of feline calicivirus (FCV) and feline herpesvirus type 1 (FHV-1) are known among the feline population worldwide (11, 25). Although it is generally assumed that these conditions are similar in Brazil, no major study regarding these viruses has been performed in that country, with the exception of several serological surveys (6, 14, 27) and an experimental study regarding pathogenicity (22). The diseases of the respiratory tract of felines are an important and recurrent problem for veterinarians and cat owners globally, and FCV and FHV-1 have been described as one of the primary causes of these clinical manifestations (2, 8). In this study, FCV, FHV-1 or both were isolated and identified from 55 cats with or without clinical signs from a total of 302 animals sampled in some cities of the Rio Grande do Sul State in the southern part of Brazil.

In the present article, FCV was isolated more often, in terms of the overall results, but its frequency was different when the groups were analyzed separately (Table 2). FCV was the primary virus isolated in most of the groups examined, although FHV-1 was the virus most frequently isolated from vaccinated cats and was also isolated slightly more frequently than FCV in cats exhibiting clinical signs of disease. A greater prevalence of FCV in comparison to FHV-1 has been reported in cats with clinical manifestations of disease as well for clinically healthy cats (2, 16), although it has been shown that FHV-1 isolation is generally related to the presence of clinical signs, whereas FCV is not (12). Furthermore, FHV-1 is the virus most commonly identified when respiratory clinical manifestations are observed (7, 13, 16, 34); in the present study, signs of respiratory disease were present in 24 out of the 25 sick cats from which FCV, FHV-1 or both were isolated. Conversely, FCV was present in samples from the three cats showing signs of oral lesions in this study (data not shown), which matches results from other studies that have associated oral ulcers more consistently with FCV infection (25, 26, 34).

The higher prevalence of FCV in comparison to FHV-1 isolated in the United Kingdom has been attributed to vaccines that began to be applied in the 1970s (12). This vaccination likely contributed to the reduction of the number of cats in the

population excreting FHV-1 but did not significantly affect the number of cats excreting FCV (12). The biology of the viruses is one characteristic that could contribute to this finding; FCV is an RNA virus with wide genetic and antigenic diversity, whereas FHV-1 is a stable DNA virus (25). In this article, the number of vaccinated cats excreting FHV-1 was higher than that excreting FCV (Table 2). However, it should be noted that there was a unique situation in this study that could cause such results. Most of the isolated FHV-1 came from breeding catteries where an outbreak of respiratory disease was occurring. Thus, many of the FHV-1-isolated cats came from the same location; furthermore, most of the vaccinated cats used in this study came from the same cattery.

In addition, FCV was the virus most frequently isolated from cats that did not show signs of disease (Table 2). As previously noted, FHV-1 is typically isolated when animals present clinic manifestations, whereas FCV is isolated when they do not (12). Again, virus biology is the most probable explanation for these results because FCV carriers excrete the virus continuously, whereas FHV-1 carriers excrete the virus only when it is reactivated (11, 13, 25). The percentages of isolation were roughly 60% for FCV and 27% for FHV-1 (Table 2). A comparison with other data shows only that FCV is more commonly isolated than FHV-1 because the prevalence detected varied as much as 15 to 25% for FCV and 0.2 to 33.3% for FHV-1 in one study (12) to 25% for FCV and 0.6% for FHV-1 in another study (3).

However, no virus was isolated from approximately 64% of the samples coming from cats showing signs of respiratory disease (Table 1). This finding could be attributed to problems with sampling and storage conditions or even cases in which cats were sampled late in the course of disease, as has been reported in other studies (2, 29). Nonetheless, there are other causes for respiratory and ocular diseases in felines other than FCV and FHV-1, including agents such as fungi, bacteria and other viruses (10). *Chlamydophila felis* is a bacteria routinely identified in cases of conjunctival disease in cats. *Bordetella* spp., which is associated with mild respiratory signs, is also commonly identified (2). Thus, cases of disease in cats from which neither virus was isolated could have other etiologic causes.

The age of the individuals was taken in account and when the results were analyzed separately for each virus, it was observed that FCV was primarily isolated from adult cats, whereas FHV-1 was primarily isolated from younger animals (results shown in Table 2). A higher prevalence of FCV in adults has been already described (34). An average age of 38 months for cats was demonstrated positive for FCV, and 29.9 months for FHV-1-positive cats (34). The results for FHV-1 also match those of previous studies, which isolated FHV-1 from 16.9% of cats between one and three months old, 8.7% from cats between 4 and 11 months old, and less than half the percentage from cats above 11 months of age (2).

As previously noted, the female/male ratio was comparable, whereas the viral isolation frequency was twice as frequent in females as in males (Table 1 and Results). The large amount of positive samples obtained from females was not an expected result. In quite a few studies performed in cat populations from numerous locations, no gender difference was observed (11, 25).

The castration status of the cats appears to play a larger role than the gender in the epidemiology because some researchers have shown a smaller number of positive spayed females and neutered males in comparison to non-castrated cats (2, 30). The higher prevalence of the virus among non-castrated cats has been attributed to the behavior of these animals; the likelihood of exposure to virus of neutered/spayed animals may be reduced because social interactions are less likely to occur (30). In the present study, it was not possible to compare the virus distribution among castrated cats because the data were not available.

The viruses were more often isolated from cats that shared a habitat (Table 1). Such results are similar to those demonstrated in surveys performed in European countries (2,

13). The high prevalence of these viruses in cats that share a living space is generally attributed to the method of transmission, which requires close contact between infected and susceptible animals (11, 25). Furthermore, another important characteristic of the Brazilian cat population is that most cats, even ones with owners, live outside. This means that cats are more exposed to infectious diseases that are transmitted by contact than the general population analyzed in surveys performed in the USA and European countries.

Taken together, the data shown here provide insight into the epidemiology of FCV and FHV-1 among the cat population in counties in southern Brazil. One point of particular concern is the detection of cats without clinical signs that excrete the viruses. Critically, this occurred even among animals that were vaccinated. Such animals could be a source of infection, particularly for kittens. In addition, because FCV is an RNA virus that varies widely, the molecular characterization of isolates of this virus is underway in our lab, and the data compiled in this article will aid in further studies relating to epidemiology and vaccine efficacy.

Both FCV and FHV are prevalent in Southern Brazil despite sporadic vaccination. Cats likely encounter these viruses at a very young age and this contributes to a complicated epidemiology for both these viruses. Of significance cats showing no clinical signs were found to be excreting virus as well as those that had previously been vaccinated against FCV and FHV. This highlights the need for continued research into these important diseases of domesticated cats.

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