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ANIMAL SCIENCE

Frequency of feline herpesvirus 1 (FHV-1) in domestic cats from Campo Grande, MS, Brazil

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Abstract: Feline herpesvirus type 1 (HVF-1) is the infectious agent of feline viral rhinotracheitis. The main clinical signs are cough, nasal and eye discharge, fever, conjunctivitis and sneezing. Although the occurrence of the virus is known in some regions of Brazil, in Campo Grande, Mato Grosso do Sul (MS), there is no epidemiological information about its frequency. Thus, this study aimed to determine the frequency of feline herpesvirus type 1 in the region, and to evaluate its possible association with clinical and epidemiological factors. Ocular, nasal and oropharyngeal swabs, and blood were collected from 152 animals and analyzed through PCR and sequencing. In addition, epidemiological and clinical data were obtained through clinical examination and anamnesis. FHV-1 was detected in samples from 84 (55.26%) animals. There was no association between infection and age or sex. However, there was a significant association between infection and nasal ($p < 0.0001$) and ocular ($p = 0.014$) discharge and sneezing ($p = 0.001$). The results demonstrate the occurrence of the virus in domestic cats in the region with a high frequency of infection. Thus, FHV-1 should be considered as a potential causal agent of upper respiratory tract disease in domestic cats from Campo Grande, MS, Brazil.

Key words: rhinotracheitis, feline respiratory complex, midwest, Brazil, domestic feline.

INTRODUCTION

The feline population in Brazil has grown considerably in recent years, at a higher growth rate than the canine population. According to estimates by the Brazilian Institute of Geography and Statistics (IBGE), the number of domestic cats in Brazil should exceed the canine population in less than ten years (IBGE 2015). Thus, feline health care should also receive greater attention.

Respiratory diseases in cats, especially infectious, are of great importance in veterinary medicine. However, due to its multifactorial character, and the absence of adequate epidemiological information, the definitive diagnosis is always difficult, and even the presumptive clinical diagnosis is compromised. Among the main agents commonly involved in respiratory tract infections of domestic cats, feline herpervirus type-1 (FHV-1) and feline calicivirus, agents of feline viral rhinotracheitis and feline viral calicivirosis, respectively, are the most frequent. These agents are responsible for the Feline Viral Respiratory Complex (FVRC) that may be present associations with *Bordetella bronchiseptica* and *Chlamydia felis* (Cai et al. 2002, Bannasch & Foley 2005, Helps et al. 2005).

FHV-1 is a double-stranded DNA virus with an icosahedral capsid, responsible for primary infection of domestic cats by oral, nasal and conjunctival routes. As with infection by other herpesviruses, neuronal infection occurs later, making the animal a lifelong latent carrier, with

episodes of reactivation and dissemination due to stress factors (Gaskell et al. 2007). While the domestic cat is the main host, FHV-1 has been isolated from numerous wild felid species, captive and free-ranging (Munson et al. 2004, Marino et al. 2021, Wu et al. 2022).

Transmission usually occurs by direct contact with nasal, eye and oral secretions from infected animals. The main clinical signs are cough, nasal and eye discharge, fever, conjunctivitis and sneezing, the latter being also responsible for transmission via aerosols (Souza & Calixto 2003, Megid et al. 2016). Vaccination is effective in attenuating clinical signs, however, it does not prevent infection (Gaskell et al. 2007).

In Brazil, FHV-1 has been identified in domestic cats from some regions, with variable frequencies (Simões 2013, Baumworcel 2014). In the Midwest region, only serological evidence has been observed in free-ranging felids (Filoni et al. 2006). In Campo Grande, Mato Grosso do Sul (MS), no epidemiological information is available. Therefore, the aim of this study was to determine the frequency of feline herpesvirus-1 (FHV-1), using molecular tools such as Polymerase Chain Reaction (PCR) and DNA sequencing in samples from domestic cats from the municipality of Campo Grande, Mato Grosso do Sul, Brazil. Also, we aimed to evaluate clinical and epidemiological factors regarding their association with infection.

MATERIALS AND METHODS

Ethical aspects and sampling

The present research received authorization from the Ethics Committee on the Use of Animals of the Universidade Federal de Mato Grosso do Sul (UFMS) (CEUA, protocol 843/2017).

A total of 152 animals (domiciled cats) were sampled. Sampling was performed randomly among the animals treated at the Veterinary Hospital of UFMS and private veterinary clinics from Campo Grande, Mato Grosso do Sul, from 2018 to 2022.

Clinical and epidemiological information were obtained through clinical examination and anamnesis from only 100 animals (at the Veterinary Hospital of UFMS). Of these, samples were collected using swab from the oropharynx, conjunctival mucous membranes (right and left), and nasal discharge, totaling four samples per animal. Of the remaining 52 animals (at the private veterinary clinics), it was not possible to perform the clinical examination and anamnesis, and only blood samples were collected (one sample per animal).

The swab samples were placed in microtubes containing 200 µL of sterile saline (0.9%) and subsequently stored at -20°C until processing. Blood was collected in vacuum tubes containing K(2) ethylenediaminetetraacetic acid (Vacuette, Greiner Bio-One, Kremsmünster, Austria).

DNA extraction

DNA extraction of the samples was performed using the method of Araújo et al. (2009), with an initial incubation step with 20 mg/ml of proteinase K (Ludwig Biotecnology, Alvorada, Rio Grande do Sul, Brazil) for 12 hours at 56°C.

After extraction, the DNA obtained from each sample was subjected to electrophoresis in a 0.8% agarose gel to verify its integrity. To evaluate the concentration, the samples were submitted to spectrophotometry with readings at 260nm and 280nm in BioPhotometer Plus (Eppendorf, Wesseling-Berzdorf, Germany). Also, to analyze the presence of PCR inhibitors, all DNA samples were submitted to PCR for the constitutive gene β-actin, as described by Wang et al. (2007). The reactions were visualized in a UV transilluminator (Bio-Rad Laboratories, Hercules, California, USA) after electrophoresis

in 1.5% agarose gel and staining with GelRed (Biotium, San Francisco, California, USA).

Detection of FHV-1

For detection of FHV-1, the PCR reaction previously developed by Sykes et al. (2001) was used. This reaction uses the HerpF (5'-GACGTGGTGAATTATCAGC-3') and HerpR (5'-CAACTAGATTTCCACCAGGA-3') primers, which delimit a 292 base pair (bp) fragment of the FHV-1 thymidine kinase (TK) gene.

Subsequently, positive samples (08) were randomly selected and submitted to DNA sequencing to confirm the PCR results. Sequencing was performed in both directions by the Sanger method (Sanger et al. 1977), in an ABI 3130 automatic sequencer (Applied Biosystems, Waltham, Massachusetts, USA), after purification of the amplified fragments with the Clean Sweep PCR purification kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA), according to manufacturer's instructions. After analyzing the electropherograms and obtaining the consensus sequence, with the aid of the BioEdit program (Hall 1999), the sequences were submitted to a search for homology with DNA sequences available in Genbank using the BLASTn tool, available at https://blast .ncbi.nlm.nih.gov/Blast.cgi. Sequences were deposited in the Genbank database.

Statistical analysis

The association between infection and the clinical and epidemiological information collected was analyzed using Fisher's exact test, with the aid of the BioEstat 5.3 program (Ayres et al. 2007).

RESULTS AND DISCUSSION

Among the 152 animals analyzed, FHV-1 DNA was found in 84, with a frequency of 55.26%. Animals that presented amplification in at least one of the samples were classified as positive.

Among the samples collected, a higher percentage of positivity was observed in nasal discharge (47.27%), followed by blood (42.85%), conjunctival swabs (27.27%) and oropharynx (25.45%). The identification of the virus in nasal, ocular and oral samples is common, even in cases of reactivation of latent infection. These biological samples are recognized as the first choice for laboratory diagnosis (Thiry et al. 2009). On the other hand, a positive blood sample is an indicator of viremia, since a brief period of viremia occurs only in cats with primary herpetic disease (Westermeyer et al. 2009). In the present study a high frequency of positivity was observed from blood samples. However, among the total samples origin, only 21 were blood, and all of them were from symptomatic animals. This feature may have favored the high frequency of positivity observed.

Positive samples whose amplicons were sequenced showed more than 98% identity with FHV-1 DNA sequences available from Genbank. The samples from the present study were deposited at Genbank under accession numbers MN326851, MN326852, OP762598, OP762599, OP762600, OP762601, OP762602 and OP762603.

The frequency of infected animals observed in the present study (55.26%) was similar to that observed by Baumworcel (2014), in the cities of Niteroi and Rio de Janeiro (57.4%), and that observed by Simões (2013) in Araçatuba, São Paulo (50%). In other regions of the world, such as the USA, FHV-1 is one of the most frequent agents (30%) in cats coming from shelters (Burns et al. 2011). And, in some regions, such as South Korea, prevalence as high as 63% has been observed (Kang & Park 2008).

In the present study, the animals were distributed into four age groups, namely < 1 year, 1 to < 3 years, 3 to < 5 years, and ≥ 5 years.

However, there was no significant association between age and infection at time of diagnosis in this study (p = 0.753). Previous studies have indicated an increased susceptibility of kittens younger than six months of age or immunocompromised individuals (Gaskell et al. 2007). Also, Grace (2018) says that all ages of cats are susceptible to FHV-1, although the virus tends to be more virulent in young kittens than adult cats. Unfortunately, there was a low number of young cats in our study to compare these data.

The same was observed in relation to gender (p = 0.213). Other researchers have also observed the non-association between FHV-1 infection and gender. Souza & Calixto (2003) and Grace (2018) observed that FHV-1 could affect both males and females equally. This observation is also supported by the results of Rodrigues (2012), among 50 cats with FHV-1 from Portugal.

Not all FHV-1 infected animals in the present study had clinical signs. Of a total of 100 animals, from which clinical information was obtained during collections, 69% had any clinical sign, while 31% were asymptomatic. Among the positive to FHV-1, the proportion of symptomatic reached 79.7%. This high proportion of symptomatic animals infected by the virus is compatible with the spread of the virus in feline populations and its pathogenic potential, alone or in association with other pathogens. The proportion of infected but asymptomatic animals (20.3% in the present study) can be explained by the fact that FHV-1, like other herpesviruses, can cause neuronal infection and a consequent long latency period, causing no harm to the host. This virus tends to cause acute lytic disease followed by periods of latency and subsequent intermittent recrudescent disease (Gould 2011).

Among the animals sampled with clinical information, there was a significant association between infection and the presence of ocular and nasal descharge, besides sneezing (Table I).

With respect to clinical signs, FHV-1 infection has been associated with the presence of signs related to the upper respiratory tract (Sykes et al. 1999, Zicola et al. 2009), despite the existence of contradictory results (Najafi et al. 2014). In the present study, a significant association was observed between FHV-1 infection and the presence of nasal discharge and sneezing, which are initial signs of upper respiratory tract involvement. In other studies, an association between infection and eye lesions has also been observed, such as conjunctivitis with the presence of serous to mucopurulent secretion, keratitis and even panophthalmia (Gould 2011, Gerriets et al. 2012). In our study, there was no case of ocular diseases other than conjunctivitis.

These results confirm that the frequency of FHV-1 in domestic cats is high in Campo Grande, Mato Grosso do Sul, and it should be considered in the etiological diagnosis of animals presenting respiratory and ocular disease.

Table I. Absolute frequency of domestic cats infected by FHV-1 in relation to the presence of nasal/eye discharge and sneezing.

Clinical sign		POSITIVE	NEGATIVE	*p-value*
Eye discharge	Present	15	6	0.014
	Absent	33	46	
Sneezing	Present	14	3	0.001
	Absent	34	49	
Nasal discharge	Present	26	5	<0.0001
	Absent	26	43	

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J.B.C. and J.T.E. contributed to the implementation of the research; C.A.N.R. and V.J.B.T. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.