SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2022v43n1p51

Experimental infection of BALB/c mice with felid alphaherpesvirus 1

Infecção experimental de camundongos BALB/c com alphaherpesvírus felino tipo 1

Débora Scopel e Silva[1]; Clarissa Caetano de Castro[2]; Fábio da Silva e Silva[1]; Fabiane Borelli Grecco[3]; Renata Nobre da Fonseca[4]*; Geferson Fischer[5]; Marcelo de Lima[5]; Silvia de Oliveira Hübner[5]

Highlights

BALB/c mice can be infected by felid alphaherpesvirus type 1 (FHV-1).
Clinical signs appeared 3 days post-infection and lasted until euthanasia.
Histological lesions and viral DNA were found in all sampled tissues.
A higher frequency of FHV-1 DNA copies was detected in the lungs.

Abstract

Felid alphaherpesvirus type 1 (FHV-1) is an important cause of respiratory and ocular diseases in cats worldwide. Mice have been widely used to study the pathogenesis of several human and animal viruses, especially herpesviruses. This study aimed to verify whether BALB/c mice are susceptible to FHV-1 infection. The animals were intranasally inoculated with FHV-1 and their clinical signs were observed from 3 days post-infection (dpi). At 10 dpi, the animals were euthanized and the lungs, liver, spleen, and kidneys were collected for histopathological examination and quantitative polymerase chain reaction. The results showed that mice were infected with FHV-1 and reproduced several features of the disease observed in its natural host. Histological lesions and viral DNA were found in all sampled tissues, with a higher frequency of FHV-1 DNA copies detected in the lungs. All mice were seroconverted to FHV-1 at 7 dpi. To our knowledge, this is the first report of experimental infection of BALB/c mice with FHV-1. Our findings demonstrate that this murine model can contribute to understanding of FHV-1 pathogenesis and may be useful for trials against this virus.

Key words: Animal model. Cats. Experimental infection. FHV-1. qPCR.

[1] PhD, Department of Preventive Veterinary Medicine, Universidade Federal de Pelotas, UFPel, Pelotas, RS, Brazil. E-mail: scopeldebora@yahoo.com.br; silvamedvet@hotmail.com
[2] Postdoctoral Researcher in the Postgraduate Program in Veterinary Medicine, Department of Animal Pathology, UFPel, Pelotas, RS, Brazil. E-mail: clarissac.decastro@gmail.com
[3] Profª. PhD, Department of Animal Pathology, UFPel, Pelotas, RS, Brazil. E-mail: fabianegrecco18@gmail.com
[4] PhD Student in the Postgraduate Program in Veterinary Medicine, Department of Preventive Veterinary Medicine, UFPel, Pelotas, RS, Brazil. E-mail: renatanobredafonseca@gmail.com
[5] Profs. PhD., Department of Preventive Veterinary Medicine, UFPel, Pelotas, RS, Brazil. E-mail: geferson.fischer@gmail.com; mdelima.ufpel@gmail.com; sohubner@yahoo.com.br
* Author for correspondence

Received: Apr. 05, 2021 - Approved: Oct. 11, 2021

Resumo

O herpesvírus felino tipo 1 (FHV-1) é um importante agente causador de doença respiratória e ocular em gatos em todo o mundo. Camundongos têm sido amplamente utilizados para estudar a patogenia de diversos vírus humanos e animais, especialmente os herpesvírus. O objetivo deste estudo foi verificar se camundongos BALB/c poderiam ser suscetíveis a infecção pelo FHV-1. Os animais foram inoculados com FHV-1 intranasalmente e sinais clínicos foram observados a partir do dia 3 após a infecção (dpi). Após dez dias da inoculação, os animais foram eutanasiados e os pulmões, fígado, baço e rins foram coletados para exame histopatológico e PCR quantitativo (qPCR). Os resultados mostraram que os camundongos foram infectados com o FHV-1 e reproduziram várias características da doença que são observadas em seu hospedeiro natural. Lesões histológicas e DNA viral foram detectadas em todos os tecidos amostrados, com maior frequência de DNA do FHV-1 nos pulmões. Todos os camundongos soroconverteram para FHV-1 aos 7 dpi. Para nosso conhecimento, este estudo é o primeiro relato de infecção experimental de camundongos BALB/c com FHV-1. Nossos resultados demonstraram que esse camundongo pode contribuir para o entendimento da patogenia do FHV-1 e pode ser um modelo útil para ensaios contra esse vírus.
Palavras-chave: FHV-1. Gatos. Infecção experimental. Modelo animal. qPCR.

Introduction

Felid alphaherpesvirus 1 (FHV-1) is classified in the order Herpesvirales, family Herpesviridae, subfamily Alphaherpesvirinae, and genus *Varicellovirus* (International Committee on Taxonomy of Viruses [ICTV], 2020). FHV-1 is a common and important cause of respiratory and ocular diseases, dermatitis, and potentially intraocular diseases in cats. FHV-1 infections are common among cats worldwide (Maes, 2012). The most common clinical manifestations of FHV-1 infection are rhinitis, conjunctivitis, and keratitis. Clinical signs in affected kittens include fever, lethargy, anorexia, rhinotracheitis with serous to mucopurulent ocular and nasal discharges, sneezing, and sometimes coughing (Cave, Dennis, Gopakumar, & Dunowska, 2014). Uncomplicated FHV-1 disease typically resolves within 1–2 weeks after infection. However, the virus establishes latency in the neuronal tissues, and FHV-1–infected cats presumably remain infected for life. Such cats may periodically have recrudescence of latent FHV-1 infection that may or may not be accompanied by clinical disease (Cave et al., 2014).

No specific drug is currently available for the treatment of FHV-1 infections. Based on the literature, veterinary clinicians mostly use antiviral drugs available for human herpesviruses to treat herpesviruses in cats (Silva et al., 2014). When a drug developed for humans infected with a herpesvirus is used to treat a cat infected with FHV-1, two major assumptions must be made: the drug is efficacious against FHV-1; and it is safe in cats (Thomasy & Maggs, 2016). Therefore, veterinary antiviral therapy research is encouraged.

Several factors must be considered when determining a drug's antiviral efficacy. Methodical investigations of *in vitro* efficacy against FHV-1, followed by pharmacokinetic and safety trials in normal cats, subsequent placebo-controlled efficacy studies in

experimentally inoculated animals, and, finally, carefully designed and monitored clinical trials in client-owned animals, is critical (Thomasy & Maggs, 2016). Although data obtained in controlled clinical trials or experimental infections in cats reflect the real efficacy of drugs against FHV-1, these studies are extremely difficult to perform. Research involving the experimental infection of cats demands suitable accommodation besides humane and ethically restrictive concerns. Thus, the use of a carefully projected experimental model could allow approaches that would not be viable in cats but could help increase our understanding of the pathogenesis of FHV-1 infection and provide evidence for new antiviral drugs. The amount of information about the genetic and biological characteristics of murine lineages makes them an attractive animal model for investigating the pathogenesis and immune mechanisms of viral clearance (Mori et al., 2012). Although experimental models have their limitations and never completely mimic the infection in their natural host, they might be useful for the initial trial and monitoring of antiviral efficacy.

There are several reports on the reproduction of herpesvirus infections in animal models, such as bovine herpesvirus 5 (BoHV-5), equid herpesvirus type 1 (EHV-1), and herpes simplex types 1 and 2 (HSV-1 and HSV-2) (Mesquita et al., 2017; Mahiet et al., 2012; Mori et al., 2012). However, to date, no studies have examined the reproduction of FHV-1 infections in animal models except in the cat itself (Rodriguez, Köhler, & Kipar, 2018). The present study aimed to evaluate the susceptibility of BALB/c mice to FHV-1 infection and the possibility of its use as an experimental model.

Materials and Methods

The laboratory strain B927 of FHV-1 (Gaskell, Dennis, Goddard, Cocker, & Wills, 1985) was propagated in Crandell-Rees Feline Kidney (CRFK number: CCL-94™; ATCC, USA) cells cultivated in Eagle's minimum essential medium (Sigma Aldrich, USA) supplemented with 10% fetal bovine serum (Gibco, USA), penicillin (Sigma Aldrich), streptomycin (Vetec, Brazil), amphotericin B (Cristália, Brazil), and enrofloxacin (Bayer, Brazil) in an incubator at 37°C with CO2 supplementation. The virus was titrated in monolayers of CRFK cells by determining the 50% endpoint titer using the Spearman-Karber method as previously described (Ramakrishnan, 2016).

Twenty-seven 4-week-old female BALB/c mice were obtained from the Central Bioterium of the Federal University of Pelotas (UFPel). All mice were acclimatized for one week before experimental manipulation and housed at 22–24°C, humidity of 40–60% and a light/dark cycle of 12/12 h in the university's animal facility. The experimental and control mice were housed separately in mini-isolators. Autoclaved water and commercial food pellets were provided *ad libitum.* All animal handling was performed in a bio-safety cabinet. The procedures were approved by the Ethical Committee for Animal Experimentation (protocol number 23110.009152/2013-59).

BALB/c mice were inoculated intranasally with a viral suspension of FHV-1 (10 µL/nostril/animal), with a titer of 10^5 tissue culture infective doses ($TCID_{50}$/100 µL). The control group (ten animals) received autoclaved distilled water in the same manner. Animals were observed daily for clinical signs of disease, body weight changes, and mortality. The animals were euthanized at ten

days post-infection (10 dpi) by an anesthetic overdose, and the organs were collected for histopathology and quantitative polymerase chain reaction (qPCR). Kidney, liver, lung, and spleen fragments were stored at -70°C for qPCR and in 10% formalin for histopathological analysis.

Blood samples were collected on days 0, 7, and 10 of the experiment, and the antibodies against FHV-1 were titrated by indirect enzyme-linked immunosorbent assay (ELISA). FHV-1 was cultured in CRFK cells; after the detection of a 90% cytopathic effect, centrifugation at 15000 rpm for 30 min was performed to clean the cell debris. ELISA plates were coated with FHV-1 (titer 10^6 $TCID_{50}$) in bicarbonate buffer (pH 9.6) overnight at 4°C. Phosphate buffered saline (PBS) with 5% non-fat dry milk was used as the blocking buffer. Serum samples diluted 1/100 in PBS containing 0.05% Tween 20 (PBS-T, pH 7.6) were incubated on plates at 37°C for 60 min. After washing with PBS-T, peroxidase-conjugated rabbit anti-immunoglobulin G (IgG) mouse immunoglobulin was added to each well and incubated at 37°C for 60 min. After five further washes with PBS-T, the substrate/chromogen solution was added and the absorbance was read at 492 nm using a microplate reader. The ELISA data are expressed as seroconversions, where the absorbance of each sample was divided by that of the serum of the same animal on day 0.

DNA extraction was performed from the collected organs using a commercial Wizard SV Genomic DNA Purification System (PROMEGA, USA) according to the manufacturer's protocol. DNA concentrations of each sample were assessed by fluorometry using a QUBIT 3.0 device with a QUBIT dsDNA Assay kit (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations.

Samples of each tissue were embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin in the Department of Pathology of the UFPel. The samples were analyzed by a veterinary pathologist who was blinded to the sample assignments of the experimental groups.

The samples were amplified by real-time quantitative PCR using the methodology of absolute quantification (Lefever et al., 2009). Primers *forward* 5′ GAC GTG AAT TAT CAG CTG AAG 3′, *reverse* 5′ AAG GTA TGG TGC GGC AAA TC 3′, and probe 5′ FAM-TGC TGC CTA TAT CAC CGC CCA CTA TCA A 3′ TAMRA were designed to amplify a conserved fragment of 77 base pairs of the thymidine kinase gene (TK) of FHV-1 (GenBank sequence KR296657.1). PCR was performed using 10 µL of 2× QuantiNova Probe PCR Master Mix (Qiagen, USA), 1 µL (450 nM) of each primer, 1 µL (250 nM) of the probe, 3 µL of RNAse-free water, and 4 µL of the extracted DNA for a final volume of 20 µL. Each cycle of amplification consisted of an initial denaturation at 95°C for 2 min, followed by 50 cycles at 95°C for 5 s and 30 s at 60oC. The cycle threshold (CT) for each sample was determined based on the number of cycles in which the fluorescence produced surpassed the limit of detection. The analysis was performed using a BIOER LineGene 9600 (Hangzhou Bioer Technology, China). For quantification, a standard curve was constructed with serial tenfold dilutions of the positive control with an initial concentration of 10^5–$10^{6,6}$ TCID50 (Feligen® CR/P vaccine, Virbac, Brazil) and a limit of detection of 2×10-1 DNA copies per microliter of extracted

sample. The R^2 value for the standard curves was ≥ 0.999, slope was -3.3, and efficiency was 101.04, reflecting the technique's high precision.

The statistical analysis was performed using Prism software (GraphPad Software, USA). The Kruskal-Wallis analytical method was used to compare viral loads among the organs of each animal. The mean absorbance values of serum from each group were subjected to two-way analysis of variance followed by Tukey's test. Values were considered statistically significant at $p<0.05$.

Results and Discussion

This study provides evidence that mice can be infected by FHV-1. According to our serological, molecular analysis, clinical sign, and histopathological findings, all animals were infected with FHV-1, except for the controls. Viral DNA was detected in all inoculated animals. The mice were seronegative before experimental inoculation with FHV-1; however, the animals were not tested for presence of FHV-1 prior to the inoculation. Increased levels of IgG were detected in the mice inoculated at 7 and 10 dpi (mean absorbance, 3.53-fold at day 7 and 4.9-fold higher at day 10 dpi than at 0 dpi). The results were considered significant ($p<0.05$) compared to the controls. Viral DNA was found on day 10 dpi in several organs of all of the inoculated mice, mainly in the lungs (100% [17/17]), followed by the liver (82.3% [14/17]), spleen (58.8% [10/17]), and kidneys (52.9% [9/17]). The mean of FHV-1 DNA copies/mL in each organ sampled was 92,469 (lung), 2,944 (liver), 1,895 (spleen), and 552 (kidney). It is important to mention that viral DNA was detected in more than one organ in some of the infected animals, and the mensuration of viral load was statistically significant among the organs ($p<0.05$).

Daily evaluations of experimentally infected mice revealed clinical signs related to upper respiratory tract disease. Uni- or bilateral mild serous ocular discharge and conjunctivitis were observed in 9 animals (52.3%); all mice presented with blepharitis (Figure 1) and blepharospasm (100%); ruffled fur was seen in eight animals (47.1%); and photophobia was recorded in 6 animals (35.3%). Clinical signs appeared at 3 dpi and lasted until euthanasia. Mice that received distilled water intranasally instead of FHV-1 showed no clinical signs. There was no difference in body weight between the infected and control groups. The incubation period described for FHV-1–infected cats varies from 2 to 6 days, which is in accordance with the presentation of clinical signs in the experimentally infected animals in our study. Clinical findings in the experimentally infected mice appeared at 3 dpi; even if mild, they were very similar to the symptoms observed in naturally (Maes, 2012) and experimentally (Rodriguez et al., 2018) FHV-1–infected cats.

Figure 1. Mice experimentally infected with felid alphaherpesvirus type 1 (FHV-1) showing (A) blepharitis manifesting as red eyes; tearing and swollen eyelids, with serous discharge at the edge of the eyelids; and (B) photophobia manifesting as partial or complete eyelid closing upon exposure to light.

FHV-1 rapidly replicates in the epithelial cells of the upper respiratory tract and the eyes and then ascends via axons of the sensory neurons to establish lifelong latency within the trigeminal ganglia (Maes, 2012). FHV-1 is primarily an upper respiratory and ocular pathogen, with only sporadic involvement of the lungs, as viremia levels are low (Maes, 2012). Unfortunately, no analysis was performed on the eyes of the experimentally infected mice. Multiple histopathological lesions were observed in the infected animals. The main histopathological lesions observed were interstitial pneumonia with mononuclear infiltrate (17/17), mild hepatitis (5/17), mild hepatic congestion (11/17), mild individual hepatocyte necrosis (3/17), lymphoid depletion in the spleen (5/17), mild interstitial nephritis (5/17), and acute tubular necrosis (5/17) as well as inclusion bodies in the lungs and liver (7/17) (Figure 2). Although not common, similar features have been described in mice experimentally infected with EHV-1 (Mori et al., 2012).

Recent studies have demonstrated low genetic diversity among FHV-1 isolates. The analyses of various isolates, vaccines, and Australian and American strains showed that the overall genomic distance between isolates was 0.093%, indicating that the FHV-1 genomes are highly conserved (Lewin et al., 2018) and that FHV-1 has less intraspecies genomic sequence variability than some other alphaherpesviruses, such as HSV-1, HSV-2, SuHV-1, and BHV-1 (Lewin et al., 2018). Therefore, the findings observed in this experimental infection with strain B927 should be similar to those of different isolates. Other studies should include strains of diverse origins to prevent erroneous and biased conclusions that could affect future studies.

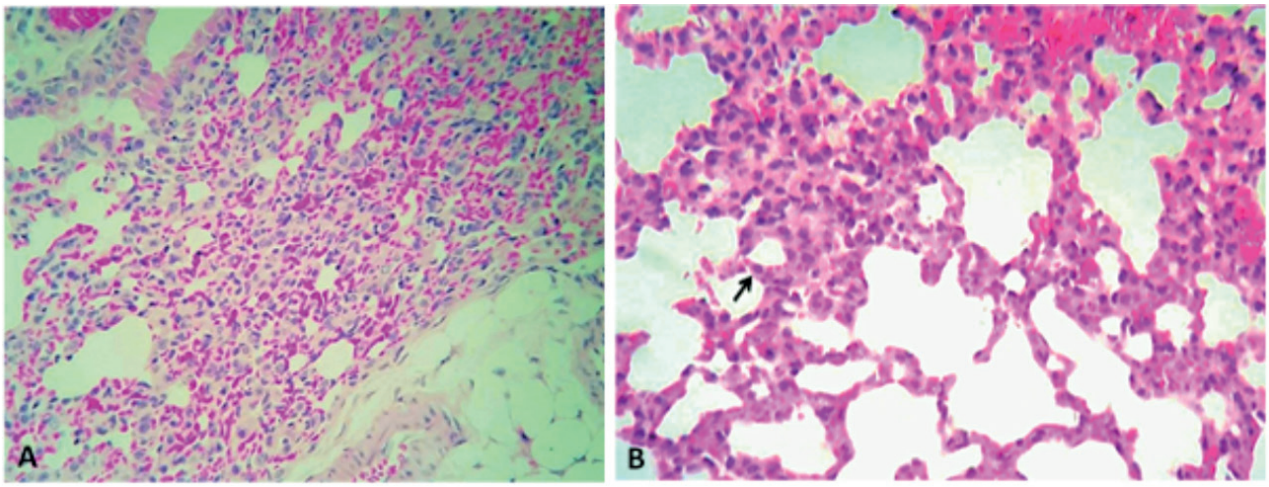

Figure 2. Histopathological findings in the lungs of a BALB/c mouse experimentally infected with felid alphaherpesvirus type 1 (FHV-1). Interstitial pneumonia evidenced by (A) moderate mononuclear infiltrate and collapse of alveolar spaces (40×, HE); and (B) proliferation of pneumocytes type II (arrow) and thickening of the alveolar septae (20×, HE). HE, hematoxylin and eosin.

Since there are no data to date regarding the use of mice as animal models for experimental FHV-1 infection, comparisons had to be made with other herpesviruses in the same genus. The intranasal inoculation of FHV-1 is a proven effective method to reproduce infection in mice since lesions and viral DNA can be found in the lungs as well as adjacent organs as demonstrated in cats (G. F. McGregor, Sheehan, & Simko, 2016) and in mice infected with EHV-1 (Mori et al., 2012). Such systemic infection in mice could also be detected for BoHV-5 (Mesquita et al., 2017), but the inoculation was performed intraperitoneally and with higher viral titers. Systemic disease is more likely to be caused by intraperitoneal or intravenous injections and, in contrast, peripheral infections have been initiated by ocular, vaginal, skin, and nasal/oral tissue inoculations (Kollias, Huneke, Wigdahl, & Jennings, 2015).

A hallmark of alphaherpesvirus biology is that acute infection is followed by lifelong persistence of the viral genome in a latent form in the nervous and lymphoid tissues (Maes, 2012). Thus, the trigeminal ganglia is another structure that would be very important in the study of FHV-1 pathogenesis and latency in mice. This is already well-established for human herpesviruses in the murine model (A. McGregor, Choi, Schachtele, & Lokensgard, 2013). In this study, the trigeminal ganglia were not collected since latency was not the major study focus.

Conclusions

Our study findings suggest that the BALB/c mouse model of FHV-1 infection reproduces several features of local and systemic diseases observed in the virus's natural host. To our knowledge, this is the first report of FHV-1 infecting mice. Although much remains to be researched and unraveled about the pathogenesis of FHV-1 in mice, the

information produced here will enable more in-depth investigations of the murine model of FHV-1 infection.

Acknowledgments

The Coordenação de Pessoal de Nível Superior (CAPES) awarded a doctoral scholarship to DS.

Strain B927 was kindly supplied by researcher Ana Cláudia Franco (UFRGS, Brazil).

Conflict of Interest Statement

The authors declare no conflicts of interest.

Ethical Approval

The study received approval from the Ethical Committee for Animal Experimentation (protocol number 23110.009152/2013-59).

References

Cave, N. J., Dennis, K., Gopakumar, G., & Dunowska, M. (2014). Effects of physiologic concentrations of L-lysine on in vitro replication of feline herpesvirus 1. *American Journal of Veterinary Research, 75*(6), 572-580. doi: 10.2460/ajvr.75.6.572

Gaskell, R. M., Dennis, P. E., Goddard, L. E., Cocker, F. M., & Wills J. M. (1985). Isolation of felid herpesvirus I from the trigeminal ganglia of latently infected cats. *Journal of General Virology, 66*(2), 391-394. doi: 10.1099/0022-1317-66-2-391

International Committee on Taxonomy of Viruses (2020). *Virus Taxonomy.* London: Microbiology Society. Retrieved from https://talk.ictvonline.org/taxonomy/

Kollias, C. M., Huneke, R. B., Wigdahl, B., & Jennings, S. R. (2015). Animal models of herpes simplex virus immunity and pathogenesis. *Journal of Neurovirology, 21*(1), 8-23. doi: 10.1007/s13365-014-0302-2

Lefever, S., Hellemans, J., Pattyn, F., Przybylski, D. R., Taylor, C., Geurts, R.,... Vandesompele, J. (2009). RDML: structured language and reporting guidelines for real-time quantitative PCR data. *Nucleic Acids Research, 37*(7), 2065-2069. doi: 10.1093/nar/gkp056

Lewin, A. C., Kolb, A. W., McLellan, G. J., Bentley, E., Bernard, K. A., Newbury, S. P., & Brandt, C. R. (2018). Genomic, recombinational and phylogenetic characterization of global feline herpesvirus 1 isolates. *Virology, 518*(14), 385-397. doi: 10.1016/j.virol.2018.03.018

Maes, R. (2012). Felid herpesvirus type 1 infection in cats: a natural host model for alphaherpesvirus pathogenesis. *ISRN Veterinary Science, 2012,* 1-14. doi: 10.5402/2012/495830

Mahiet, C., Ergani, A., Huot, N., Alende, N., Azough, A., Salvaire, F.,... Borradeau, S. (2012). Structural variability of the herpes simplex virus 1 genome in vitro and in vivo. *Journal of Virology, 86*(16), 8592-8601. doi: 10.1128/JVI.00223-12

McGregor, A., Choi, K. Y., Schachtele, S. J., & Lokensgard, J. R. (2013). Human herpesviruses and animal models. In P. Michael Conn (Ed.). *Animal models for the study of human diseases* (pp. 905-925). San Diego, CA: Elsevier.

McGregor, G. F., Sheehan, K., & Simko, E. (2016). Pneumonia and gastritis in a cat caused by feline herpesvirus-1. *Canadian Veterinary Journal, 57*(2), 147-150. PMID: 26834264

Mesquita, L. P., Costa, R. C., Fusuma, M. M., Bruhn, F. R. P., Mori, E., Pituco, E. M.,... Maiorka, P. C. (2017). Susceptibility of mice to bovine herpesvirus type 5 infection in the central nervous system. *Veterinary Research Communications, 41*(4), 279-288. doi: 10.1007/s11259-017-9699-4

Mori, C. M. C., Mori, E., Favaro, L. L., Santos, C. R., Lara, M. C. C. S. H., Villalobos, E. M. C.,... Maiorka, P. C. (2012). Equid herpesvirus type-1 exhibits neurotropism and neurovirulence in a mouse model. *Journal of Comparative Pathology, 146*(2-3), 202-210. doi: 10.1016/j.jcpa.2011.04.003

Ramakrishnan, M. A. (2016). Determination of 50% endpoint titer using a simple formula. *World Journal of Virology, 5,* 85-86. doi: 10.5501/wjv.v5.i2.85

Rodriguez, M., Köhler, K., & Kipar A. (2018). Calicivirus co-infections in herpesvirus pneumonia in kittens. *The Veterinary Journal, 236,* 1-3. doi: 10.1016/j.tvjl.2018.04.004

Silva, D. S., Castro, C. C., Silva, F. S., Fernandes, M. H. V., Lorenzini, F., Cordeiro, J. M. C.,... Hübner, S. O. (2014). Perspectivas terapêuticas no tratamento das infecções pelo herpesvírus tipo 1. *Revista Clínica Veterinária, 19*(109), 36-44.

Thomasy, S. M., & Maggs, D. J. (2016). A review of antiviral drugs and other compounds with activity against feline herpesvirus type 1. *Veterinary Ophthalmology, 19*(1), 119-130. doi: 10.1111/vop.12375