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MICROBIOLOGY

Feline herpesvirus 1 viral load related to environmental factors in sheltered cats

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ABSTRACT: *Shelter environment stress factors are related to FHV-1 viral reactivation. However, comparisons between conjunctival viral load and environmental factors have not been commonly evaluated. The aim of this study was to correlate FHV-1 viral load in domestic cats with and without clinical signs of conjunctivitis to shelter design in order to use FHV-1 viral load as a parameter of "health management". Cats from four different shelters underwent an ophthalmological examination. Samples were collected by rolling a DNA/RNAse-free cytobrush over the ventral conjunctival fornix and were stored in 1.5 mL sterile microtubes in 500 µL of Eagle's minimum essential medium and kept at 4 ºC. Molecular procedures were performed up to 48 hours after collection. Different routines regarding new arrivals were directly related to FHV-1 viral load. Shelters where new arrivals occurred on daily basis had the highest viral load ($2.69x10^8$ copies/µL), while those shelters where new arrivals had not occurred in the few months prior to the beginning of the study had the lowest rate ($1.63x10^3$ copies/µL). Environmental factors directly influenced FHV-1 DNA viral load. This study highlighted the need to improve the management approach in the animal shelter environment to reduce stressful situations responsible for FHV-1 reactivation and higher viral load quantification.*
Key words: *FHV-1, conjunctivitis, shelter medicine, qPCR, animal shelter.*

Relação entre carga viral de herpesvirus felino 1 e fatores causadores de estresse em felinos de abrigo

RESUMO: *No ambiente do abrigo encontram-se fatores que geram estresse nos animais que ali residem. Esses fatores acabam por provocar a reativação do FHV-1. No entanto, comparações entre carga viral conjuntival e fatores ambientais não foram ainda avaliadas. Objetivo deste estudo foi correlacionar a carga viral de FHV-1 em felinos domésticos com e sem sinais clínicos de conjuntivite com as características dos abrigos. Assim, pode-se usar carga viral de FHV-1 como parâmetro de sanidade. Todos os gatos foram submetidos a exame clínico oftalmológico. Amostras foram coletadas com uso de escova citológica, acondicionadas em microtubos estéreis de 1,5mL contendo 500 µL de meio Eagle essencial mínimo e mantidas em 4 ºC. Análises moleculares foram realizadas no prazo de 48 horas após coleta. A rotina de entrada de novos animais estava diretamente relacionada a carga viral de FHV-1. Abrigos com entrada diária apresentaram carga viral maior ($2.69x10^8$ cópias/µL), do que abrigo onde novos animais não chegaram nos meses que antecederam a coleta ($1.63x10^3$ cópias/µL). Fatores ambientais influenciam diretamente carga viral de FHV-1. Esse estudo evidencia a necessidade de aprimorar o sistema de manejo dos abrigos de forma a reduzir situações de estresse responsáveis pela reativação de FHV-1 e consequente aumento na carga viral.*
Palavras-chave: *FHV-1, conjuntivite, medicina de abrigo, qPCR, abrigo animal.*

INTRODUCTION

Animal shelters house a large number of animals from different species, have a high turnover of animals, high population density, and a mixture of cats from different source colonies (FINKA et al., 2014; TANAKA et al., 2017). These characteristics have long been recognized as crucially important determinants for the risk of acquiring contagious infectious diseases (TANAKA et al., 2012; PESAVENTO & MURPHY, 2014). Domestic cats are common sheltered animals and conjunctivitis is one of the most frequent diseases at sites with large population concentrations (DAVIS-WURZLER, 2014; TANAKA et al., 2017).

Feline herpesvirus 1 (FHV-1) is one of the pathogens responsible for conjunctivitis (BAUMWORCEL et al., 2017; FERNANDEZ et al., 2017) FCV, Mycoplasma felis, and Chlamydophila felis. However, infected animals establish latency;, ;therefore, the first infection may result in reactivation in stressful situations (MÖSTL et al., 2013). The

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particular conditions to which these animals are submitted in shelters generate a stress situation that facilitates the reactivation of FHV-1 and; consequently, the spread of infection to susceptible animals (STELLA & CRONEY, 2016).

For these reasons, understanding the importance of stress in shelter cats and being able to identify and mitigate stress whenever possible is critical for maintaining healthy shelter cat populations (AMAT et al., 2016). Thus, determining which shelter characteristics influence FHV-1 viral load is essential for improving the design and management of shelters as well as kitten welfare. The characteristics analyzed in the present study were parameters previous established by the Association of Shelters Veterinarians and included building design adaptation, temperature control, presence of non-porous surfaces, proximity to other species, mode of housing animal (caged or not), distance between cages (>45cm), minimal spacing (60 cm) between litterbox, resting place and food, and presence of an in-house veterinarian.

The aim of this study was to correlate FHV-1 viral load in domestic cats with shelter design (shelter environmental factors, animal housing characteristics, and presence of an in-house veterinarian) in order to use FHV-1 viral load as a parameter of "health management".

MATERIALS AND METHODS

Study locations - shelters

Shelter enrollment in this study was convenience based and criteria for inclusion were permission from the shelter manager, ability to collect required data, and distance from the shelter and laboratory not exceeding 70 kilometers. Four different shelters in Rio de Janeiro, Brazil were included in this study. Shelters were designated as A, B, C, and D according to the order in which they were visited at the beginning of the study. In all four shelters, cleaning methods were similar, and they all used benzalkonium chloride 2%. Each shelter had its own responsible technical team.

New arrivals occured differently in each shelter. In shelter A there were no arrivals in prior months to our visit. In shelter B, new arrivals occured on weekly basis. In shelter C new arrivals occured on daily basis while in shelter D new arrivals occured randomly. The animal housing characteristics and shelter environmental factors are listed in table 1.

Study population

This research was conducted with UFF Ethics Committee of Animal Research approval (CEUA Nº 330/2013 and CEUA N º 708/2016). A total of 70 intact kittens (28 males and 42 females) underwent an ophthalmological examination by the same veterinarian (NB) who swabbed all cats from this study between September 2015 and September 2016. The inclusion criteria were: kittens up to 12 months old without previous use of systemic or topic ocular drugs. All kittens from each one of the four shelters visited that matched the inclusion criteria were included. Topical anesthetics and fluorescein staining were not used during the ophthalmological examination because these compounds can affect the sensitivity of qPCR methods (GOULD, 2011; HORZINEK et al., 2013). A defined set of criteria for the conjunctivitis score system was adapted from a previously established one (HARTMANN et al., 2010) and was used in all shelters. Score 0 standed for no clinical signs of conjunctivitis, score 1 for mild conjunctival hyperaemia, score 2 for moderate

Table 1 - Four shelters in Rio de Janeiro, Brazil were assessed in the present study. Shelter characteristics including environmental factors, animal housing characteristics and presence of an in-house veterinarian.

Shelter characteristic	A	B	C	D
Building adapted to be a shelter	Yes	No	No	Yes
Temperature control	No	Yes	Yes	No
Easily cleaned	No	No	Yes	No
Proximity to other species	No	Yes	Yes	No
Caged animals	No	Yes	Yes	No
Distance between cages > 45 cm?	NA	Yes	No	NA
Minimal spacing (60 cm) between litterbox, resting place, and food recommended by the Association of Shelter Veterinarians respected	No	No	Yes	No
In-house veterinarian	No	Yes	Yes	No

NA - not applicable.

conjunctival hyperaemia and mild chemosis, while score 3 for severe conjunctival hyperaemia and moderate to severe chemosis. All animals housed in the same shelter that matched inclusion criterias were added to this study. Cats without conjunctivitis were swabbed first, but at the same visit as those with conjunctivitis. A total of 41 kittens had clinical signs of conjunctivitis. Twenty-nine kittens without clinical signs of conjunctivitis and without any previous history of ocular disease with the same age as those with clinical signs were also swabbed. In shelter A there were 14 cats in total but only eight of them matched the inclusion criteria. In shelter B, there were 22 cats in total but only 17 of them matched the inclusion criteria. In shelter C, there were around 150 cats in total, but only 31 of them were in an accessible area and matched the inclusion criteria. For last, in shelter D there were 24 cats in total, 14 of them matching the inclusion criteria.

DNA extraction and quantitative PCR (qPCR)

From the conjunctival samples, a pool was made, combining the right eye sample with the left eye sample from the same animal. Aliquots were vortexed (10.000X per 5 min) to assure consistente conditions for DNA extractions and subsequente analysis of FHV-1. The DNA extraction was performed using a PureLink spin column-based kit for genomic DNA (Invitrogen) according to the manufacturer's instructions. qPCR was performed to detect and quantify the FHV-1 target gene TK, according to previously described conditions (HELPS et al., 2003). A cycle threshold (Ct) <38 was considered to indicate a clear positive result and it was standardized automatically by *StepOne*™. (Applied Biosystems). All tests included a negative control containing Milli-Q water. The Felocell CVR-C (Zoetis) vaccine was used as the positive control.

Briefly, the 25-µL real-time PCR reaction contained 12.5 µL of a TaqMan® Universal Master Mix ll with UNG ; 1.0 µL (10 µM) of each primer (Primer F – GGACAGCATAAAAGCGATTG; Primer R – AACGTGAACAACGACGCAG) with 74bp ; 0.5 µL (µM) of probee (FAM-QSY); 5.0µL of sterile water; and 10 µL of extracted template DNA. All reactions were performed on a StepOne (Applied Biosystems™) thermocycler for the following conditions: 2 minutes at 50 °C, 10 minutes at 95 °C, and then 40 cycles (each cycle consisted of a denaturation step [15 seconds at 95 °C] followed by an annealing step [1 minute at 60 °C]).

Realtime test was standardized according to MIQE guidelines (BUSTIN et al., 2009). All samples, standard curve dilutions positive and negative controls were tested in duplicates. The method used to quantify FHV-1 DNA viral load was using a synthetic curve as standard curve.

The synthetic curve was drawn through *GeneArt* program. The gene block was synthesized on a 2448bp plasmid DNA segment with a 89bp insert. The sequence that encodes for TK gene and used as template was available from GenBank (JX628812).

For dilution of the synthetic standard curve, the 5 µg of lyophilized plamidial DNA were diluted in 200 µL of TRIS EDTA solution quantified at (0.016 ng/µL). The concentration reported in ng/µL was transformed into genome copy numener per microliter. Serial dilutions were made at base 10, for the standard qPCR curve. The Ct value of each dilution was then compared to target copy number in order to establish a standard curve to be used. Dilutions were 10^0-10^8 copies/µL.

Statistical analyses

The mean values of viral load were initially converted to log 10 in order to decrease the variation as a function of the mean and to obtain a normal distribution. Analysis of variance (ANOVA) was used to compare mean viral loads in animals with and without clinical signs of conjunctivitis and to compare shelter characteristics with viral loads. *P* values <0.05 were considered statistically significant.

RESULTS

Animals were 2–10 months old (mean age of 4.7 months ± 2.7 months). The FHV-1 DNA was detected in equal prevalence regardless of gender, in all shelters; although, some characteristics had more influence on viral load than others. Not all parameters established by the Association of Shelters Veterinarians (ASV) and analyzed in this study, were observed at four shelters. Parameters analyzed according to FHV-1 viral loads are summarized in table 2. Mean viral load according to new arrivals system is listed in table 3.

In shelter A all eight animals tested positive in qPCR assay for FHV-1 DNA. In shelter B out of 17 animals tested, only two of them were negative. In shelter C, out of 31 only one was negative while in shelter D, four out of 14 were negative.

The FHV-1 DNA was detected in samples obtained from 26 (89.6%) of the 29 asymptomatic cats. The FHV-1 viral load was measured and ranged from $9.41x10^0$–$2.87x10^4$ copies/µL (median $6.70 x10^1$ copies/µL). Of the 41 symptomatic cats, FHV-1 DNA was detected in 37 samples (90.2%). FHV-1

viral load was measured and ranged from $6.81x10^0$ –$1.83x10^8$ copies/µL (median $6.13x10^2$ copies/µL). A significant ($P<0.05$) difference in FHV-1 DNA viral load was detected between cats with and without clinical signs of conjunctivitis.

DISCUSSION

The prevention of infectious disease is one of the main objectives of shelter veterinary medicine. To reduce FHV-1 infection, it is important to determine the potential risk factors that increase FHV-1 DNA viral load in sheltered cats.

This was the first study to quantify FHV-1 DNA viral load in cats from different shelters in Brazil. The prevalence of FHV-1 in cats from shelters was determined as being up to 90% using the qPCR assay. The qPCR assay used Thymidine Kinase (TK) gene as the target gene. The TK is part of the kinases family of enzimes with high degree of conservation between species and inside the same specie (SOLAROLI et al., 2006) feline herpesvirus TK (FHV-TK. The use of this kind or target gene reduces the incidence of false results.

The detection of an extremely high number of cats positive for FHV-1 was a surprise since a previous study in a similar population using PCR detected FHV-1 DNA in 57.4% of samples (BAUMWORCEL et al., 2017)FCV, Mycoplasma felis, and Chlamydophila felis. This difference could be explained by the sensitivity of the qPCR technique (LITSTER et al., 2015)for a total of 22 study cats. Combined conjunctival and oropharyngeal swab specimens were tested by quantitative real-time PCR (qPCR.

There was no difference on FHV-1 viral load according to sex. The detection of FHV-1 regardless of gender confirms previous studies (GRAHAM; et al. 2017; MAAZI et al., 2016).

A statistical difference in detection FHV-1 DNA between animals with and without clinical signs of conjunctivitis was observed. However, a minimum infectious dose has not been determined for FHV-1 infection, any viral load can be considered potentially relevant. The high level of positive asymptomatic animals indicated that they cannot be excluded from suspected FHV-1 infection. Clinical screening alone is insufficient for appropriate control of FHV-1 infection in shelter kittens and qPCR should be used as a diagnostic tool. Nevertheless, it is not possible to rule out that potential confounding between health status and arrival time may have introduced bias into this analysis.

Shelter spaces tend not to be completely welcoming, and a minimum level of stress is inevitable. Feline herpesvirus is directly reactivated by stress (GOURKOW & PHILLIPS, 2015) and is a common cause of infectious diseases in shelters. To understand the importance of stress in shelter cats and be able to identify and mitigate

Table 2 - Shelter characteristics including environmental factors, animal housing characteristics and presence of an in-house veterinarian and their respective statistical significance in relation to FHV-1 DNA viral load. All kittens from each one of the four shelters visited that matched the inclusion criteria were included in mean viral load (copies/µL) calculation.

Shelter characteristic	*Yes* Mean viral load ± SD (copies/µL)	*No* Mean viral load ± SD (copies/µL)	*P-value*
Building adapted to be a shelter	$1.90 \times 10^{10} \pm 8.63 \times 10^6$	$8.15 \times 10^9 \pm 1.93 \times 10^7$	$P<0.01$
Temperature control	$8.15 \times 10^9 \pm 1.93 \times 10^7$	$1.90 \times 10^{10} \pm 8.63 \times 10^6$	$P<0.01$
Easily cleaned	$5.14 \times 10^8 \pm 2.06 \times 10^7$	$1.90 \times 10^{10} \pm 2.06 \times 10^7$	$P<0.01$
Proximity to other species	$1.90 \times 10^{10} \pm 2.06 \times 10^7$	$8.15 \times 10^9 \pm 1.93 \times 10^7$	$P<0.001$
Caged animals	$1.90 \times 10^{10} \pm 2.06 \times 10^7$	$8.15 \times 10^9 \pm 1.93 \times 10^7$	$P<0.01$
Distance between cages > 45 cm	$1.75 \times 10^9 \pm 1.49 \times 10^7$	$1.90 \times 10^{10} \pm 2.06 \times 10^7$	$P>0.05$
Minimal spacing (60 cm) between litterbox, resting place and food recommended by the Association of Shelter Veterinarians respected	$5.14 \times 10^8 . \pm 1.25 \times 10^7$	$1.90 \times 10^{10} \pm 2.06 \times 10^7$	$P<0.001$
In-house veterinarian	$8.15 \times 10^9 \pm 1.93 \times 10^7$	$5.14 \times 10^8 . \pm 1.25 \times 10^7$	$P<0.001$

SD – Standard Deviation

Table 3 - Number of cats analyzed in each shelter visited in this study and the mean FHV-1 viral load according to each shelter and their respective new arrivals system.

Shelter	Total number of cats in the shelter	Number of cats that matched inclusion criterias	Mean viral load ± SD (copies/µL)
A (no new arrivals)	14	8	$1,63x10^3 \pm 1,29x10^3$
B (weekly basis)	22	17	$3,87x10^6 \pm 1,59x10^7$
C (daily basis)	+/- 150	31	$2,69x10^8 \pm 1,04x10^9$
D (randomly)	24	14	$7,03x10^6 \pm 2,61x10^7$

SD – Standard Deviation.

stress whenever possible is critical for maintaining healthy shelter cat populations (AMAT et al., 2016). Thus, to be able to point out which shelter characteristics influence FHV-1 viral load is essential for improving the design and management of shelters as well as kitten welfare.

Our results revealed that environmental risk factors including building design adaptation, temperature control, and presence of non-porous surfaces cannot be considered separately. Shelters that were not originally built to be shelters had higher viral load than those that were planned and built as shelters. However, decreasing population density in adapted shelters could contribute to decrease stress and, consequently, lower viral loads. A smaller number of animals could contribute to better quality housing, as previously suggested regarding capacity for care (C4C) (KARSTEN et al., 2017). The frequent introduction of new animals might be an explanation for virus reactivation. Different routines regarding new arrivals were directly related to FHV-1 viral load. Shelters where new arrivals occurred more frequently had the highest viral load. A bias of this study might be when after shelter arrival samples were collected, as this could impact level of stress and consequently increase FHV-1 DNA viral load.

Although, a minimum distance of 45 cm between cages has been stipulated by the ASV (NEWBURY et al., 2010), this environmental factor did not contribute to higher viral loads when this distance was not observed. The lack of difference could be explained by the distance viral load particles migh travel in sneezes, that varies from 1-2 meters (POVEY; JOHNSON, 1970). The distance between cages probably has to be greater than one meter for this factor to have an effect. Our study suggested that caged animals have higher viral loads than uncaged ones. Uncaged animals have a better quality of life than caged ones (WAGNER et al., 2018). Being caged is a stressful factor and could reflect the recrudescence of latent FHV-1 infection as a result of stress.

The proximity to other animal species was related to higher viral loads reported on shelters B and C. Noises and odors from other species is not well tolerated by sheltered animais (STELLA; CRONEY, 2016)free-roaming (70 million. It increases stress in cats (NEWBURY et al., 2010). Our results reinforce the necessity for an appropriate acoustic environment for good animal health and welfare.

The presence of an in-house veterinarian did not contribute to lower viral loads. An effective FHV-1 infection control program requires more than solely veterinary procedures. Non-medical factors, such as the architectural design of the shelter and an appropriate housing system should also be considered in addition to an in-house veterinarian, as previously discussed (FINKA et al., 2014).

Some stress-related issues can be solved, and others reduced. In one way or another, this approach may decrease FHV-1 reactivation and viral load quantification. The information provided here; although, a bigger sample data would be more representative, may be extrapolated to other shelters and may help to improve the management approach in animal shelters. In this manner, it could reduce stress situations that can contribute to FHV-1 reactivation. It is important to emphasize that there is no standard protocol to be adopted generally by all shelters. It should be adjusted to each shelter budget and structural conditions.

CONCLUSION

All environmental stress factors analyzed in this study, except for the distance between cages, were directly related to higher viral loads of FHV-1. This study highlighted the need to improve the management approach in the animal shelter

environment in order to reduce stress situations responsible for FHV-1 reactivation and higher viral load quantification.

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AUTHOR´S CONTRIBUTION

The idea of the paper was conceived by Natasha Baumworcel, Ana Maria Barros Soares, Tatiana Xavier de Castro and Nadia Regina Pereira Almosny; Experiments were designed by Natasha Baumworcel and Tatiana Xavier de Castro and performed by Natasha Baumworcel, Joylson de Jesus Pereira. Data were analyzed by Guilherme Nunes de Souza.The paper was written by Natasha Baumworcel, Tatiana Xavier de Castro e Ana Maria Barros Soares.

REFERENCES

AMAT, M. et al. Stress in owned cats: behavioural changes and welfare implications. **Journal of Feline Medicine and Surgery**, v.18, n.8, p.577–586, 2016. Available from: <http://journals.sagepub.com/doi/10.1177/1098612X15590867>. Accessed: Dec. 4, 2017. doi: 10.1177/1098612X15590867.

BAUMWORCEL, N. et al. Correlation between clinical signs of feline conjunctivitis and molecular detection of felid herpesvirus-1, feline calicivirus, chlamydophila felis and mycoplasma felis in cats from shelters in Rio de Janeiro. **Braz. J. Vet. Res. Anim. Sci., São Paulo**, v.54, n.1, p.18–26, 2017. Available from: <http://dx.doi.org/10.11606/issn.1678-4456.bjvras.2017.104588>. Accessed: Nov. 17, 2014. doi: 10.11606/issn.1678-4456.bjvras.2017.104588.

BUSTIN, S. et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. **Clinical chemistry,** v.55, n.4, p.611-22, 2009. Available from: <http://dx.doi.org/10.1373/clinchem.2008.112797>. Accessed: Nov. 17, 2014. doi: 10.1373/clinchem.2008.112797.

DAVIS-WURZLER, G. M. 2013 Update on Current Vaccination Strategies in Puppies and Kittens. **The Veterinary clinics of North America. Small animal practice**, v.44, n.2, p.235–63, 2014. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24580989>. Accessed: May, 6, 2014. doi: 10.1016/j.cvsm.2013.11.006.

FERNANDEZ, M. et al. Prevalence of feline herpesvirus-1 , feline calicivirus , Chlamydophila felis and Mycoplasma felis DNA and associated risk factors in cats in Spain with upper respiratory tract disease , conjunctivitis and / or gingivostomatitis. **Journal of feline medicine and surgery**, v.19, n.4, p.461–469, 2017. Available from: <https://doi.org/10.1177/1098612X16634387>. Accessed: Aug. 30, 2016. doi: 10.1177/1098612X16634387.

FINKA, L. et al. A critically appraised topic (CAT) to compare the effects of single and multi-cat housing on physiological and behavioural measures of stress in domestic cats in confined environments A critically appraised topic (CAT) to compare the effects of single. **BMC Veterinary Research**, v.10, n.73, p.2–11, 2014. Available from: <http://www.biomedcentral.com/1746-6148/10/73>. Accessed: Nov. 8, 2017. doi: 10.1186/1746-6148-10-73.

GOULD, D. Feline herpesvirus-1: ocular manifestations, diagnosis and treatment options. **Journal of feline medicine and surgery**, v.13, n.5, p.333–46, 2011. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21515221>. Accessed: Oct. 14, 2013. doi: 10.1371/journal.pone.0190140.

GOURKOW, N.; PHILLIPS, C. J. C. Effect of interactions with humans on behaviour, mucosal immunity and upper respiratory disease of shelter cats rated as contented on arrival. **Preventive Veterinary Medicine**, v.121, n.3–4, p.288–296, 2015. Available from: <http://dx.doi.org/10.1016/j.prevetmed.2015.07.013>. Accessed: Nov. 23, 2016. doi: 10.1016/j.prevetmed.2015.07.013.

GRAHAM, K. et al. Feline corneal sequestra: outcome of corneoconjunctival transposition in 97 cats (109 eyes). **Journal of Feline Medicine and Surgery**, v.19, n.6, p.710–716, 2017. Available from: <http://journals.sagepub.com/doi/10.1177/1098612X16645144>. Accessed: Jul. 6, 2017. doi: 10.1177/1098612X16645144.

HARTMANN, A. D. et al. Detection of bacterial and viral organisms from the conjunctiva of cats with conjunctivitis and upper respiratory tract disease. **Journal of feline medicine and surgery**, v.12, n.10, p.775–82, 2010. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20817584>. Accessed: Oct. 4, 2013. doi: 10.1016/j.jfms.2010.06.001.

HELPS, C. et al. Detection of Chlamydophila felis and Feline Herpesvirus by multiplex REal-Time PCR Analysis. **Journal of Clinical Microbiology**, v.41, n.6, p.2734–2736, 2003. Available from: <https://doi.org/10.1128/JCM.41.6.2734–2736.2003>. Accessed: Oct. 17, 2014. doi: 10.1128/JCM.41.6.2734–2736.2003.

HORZINEK, M. C. et al. Update of the 2009 guidelines on prevention and management of feline infectious diseases. **Journal of Feline Medicine and Surgery**, v.15, n.7, p.530–539, 2013. Available from: <http://journals.sagepub.com/doi/10.1177/1098612X13489208>. Accessed: May, 16, 2017. doi: 10.1177/1098612X13489208.

KARSTEN, C. L. et al. An observational study of the relationship between Capacity for Care as an animal shelter management model and cat health, adoption and death in three animal shelters. **The Veterinary Journal**, v.227, p.15–22, 2017. Available from: <http://dx.doi.org/10.1016/j.tvjl.2017.08.003>. Accessed: Nov. 8, 2017. doi: 10.1016/j.tvjl.2017.08.003.

LITSTER, A. et al. Detection of feline upper respiratory tract disease pathogens using a commercially available real-time PCR test. **The Veterinary Journal**, v.206, p.149–153, 2015. Available from: <http://dx.doi.org/10.1016/j.tvjl.2015.08.001>. Accessed: Jan. 31, 2016. doi: 10.1016/j.tvjl.2015.08.001.

MAAZI, N. et al. Occurrence of Chlamydophila felis , feline herpesvirus 1 and calcivirus in domestic cats of Iran. Iranian **Journal of Microbiology**, v.8, n.5, p.312–315, 2016. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/28149490>. Accessed: May, 4, 2017.

MÖSTL, K.et al. Prevention of infectious diseases in cat shelters: ABCD guidelines. **Journal of feline medicine and surgery**,

v.15, n.7, p.546–54, 2013. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23813812>. Accessed: Jun 15, 2014. doi: 10.1177/1098612X13489210.

NEWBURY, S. et al. Guidelines for Standards of Care in Animal Shelters. **The Association of Shelter Veterinarians**, p. 1–67, 2010. Available from: <https://www.sheltervet.org/assets/docs/shelter-standards-oct2011-wforward.pdf>. Accessed: Aug 28, 2017.

PESAVENTO, P. A.; MURPHY, B. G. Common and Emerging Infectious Diseases in the Animal Shelter. **Veterinary pathology**, v.51, n.2, p.478–91, 2014. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24265288>. Accessed: Jan. 21, 2014. doi: 10.1177/0300985813511129.

POVEY, R.; JOHNSON, R. Observations on the epidemiology and control of viral respi- ratory disease in cats. **Journal of small animal practice**, v.11, p.485–494, 1970. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/4851977>. Accessed: Jun. 15, 2014.

SOLAROLI, N. et al. Substrate specificity of three viral thymidine kinases (TK): vaccinia virus TK, feline herpesvirus TK, and canine herpesvirus TK. **Nucleosides, nucleotides & nucleic acids**, v.25, n.9–11, p.1189–1192, 2006. Available from: <http://dx.doi.org/10.1080/15257770600894451>. Accessed: Mar. 8, 2016. doi: 10.1080/15257770600894451.

STELLA, J. L.; CRONEY, C. Environmental Aspects of Domestic Cat Care and Management: Implications for Cat Welfare. **Scientific World Journal**, p.1–7, 2016. Available from: <http://dx.doi.org/10.1155/2016/6296315>. Accessed: Jan. 18, 2017. doi: 10.1155/2016/6296315.

TANAKA, A et al. Epidemiological evaluation of cat health at a first-response animal shelter in Fukushima , following the Great East Japan Earthquakes of 2011. **PloS one**, v.12, n.3, p.1–15, 2017. Available from: <https://doi.org/10.1371/journal.pone.0174406>. Accessed: Nov. 8, 2017. doi: 10.1371/journal.pone.0174406.

TANAKA, A. et al. Associations among weight loss, stressand upper respiratory tract infection in shelter cats. **Javma-Journal of the American Veterinary Medical Association**, v.240, n.5, p.570–576, 2012. Available from: <https://www.ncbi.nlm.nih.gov/pubmed/22332626>. Accessed: Nov. 8, 2017. doi: 10.2460/javma.240.5.570.

WAGNER, D.C. et al. Cage size, movement in and out of housing during daily care, and other environmental and population health risk factors for feline upper respiratory disease in nine North American animal shelters. **PLoS ONE**, v.13, n.1, p.1–15, 2018. Available from: <https://doi.org/ 10.1371/journal.pone.0190140>. Accessed: Feb. 5, 2018. doi: 10.1371/journal.pone.0190140.