Aggressive behavior in cats naturally infected with Feline immunodeficiency virus (FIV) and its interaction with FIV disease progression

Amin Azadian[1*], Mohsen Hanifeh[1] and Masoumeh Firouzamandi[2]

[1]*Department of Veterinary Clinical Science, Division of Small Animal Internal Medicine, Faculty of Veterinary Medicine, University of Tabriz, Islamic Republic of Iran.*
[2]*Department of Veterinary Pathobiology, Division of Molecular Biology, Faculty of Veterinary Medicine, University of Tabriz, Islamic Republic of Iran.*

[*]*Corresponding author at: Faculty of Veterinary Science, University of Tabriz, Tabriz, Iran.*
E-mail: a.azadian90@ms.tabrizu.ac.ir.

Veterinaria Italiana 2020, **56** (3), 169-176. doi: 10.12834/VetIt.1795.9466.3
Accepted: 18.06.2019 | Available on line: 31.12.2020

Keywords
Feline immunodeficiency virus,
Cat,
Aggression,
DNA,
PCR.

Summary
A study was undertaken to determine the possible interaction between aggressive behavior and Feline immunodeficiency virus (FIV) disease progression based on semi-quantitative viral load levels and health status in naturally FIV-infected cats. FIV status was determined in ninety-six owned and stray cats, using nested polymerase chain reaction (PCR). Aggressive tendencies were assessed based on observation and the cats' demeanor as determined by the owners and shelter caretakers. Results showed that forty-seven cats (49%) were PCR-positive for FIV infection and all aggressive cats were FIV-positive (100%). FIV infection was significantly linked to extreme aggressive tendencies and the extremely aggressive FIV-infected cats were more likely to have an unhealthy status compared to the non-aggressive individuals ($p = 0.022$). There was also a significant difference ($p = 0.012$) in the mean Cycle threshold (Ct) values between the aggressive and non-aggressive FIV-infected cats and also between the unhealthy FIV-infected cats with extreme aggressive tendencies and the healthy FIV-infected individuals without aggression ($p = 0.001$). Accordingly, results indicated that parameters associated with FIV disease progression are directly linked to aggression. The possible impact of FIV on the behavioral pattern of naturally infected cats should not be underestimated. However, there is an urgent need to conduct more experiments to support the assumptions about the possible exacerbation of aggression tendencies in naturally FIV-infected cats following the direct effect of FIV through the course of the infection.

Introduction

Feline immunodeficiency virus (FIV) disease is one of the most important infectious diseases in domestic cats caused by a virus of the genus *Lentivirus* within the family of *Retroviridae* (Pedersen *et al.* 1987, Miyazawa *et al.* 1994). Pathogenesis of FIV infection includes three different phases. The transient phase is the primary stage of the infection. During this phase the virus replicates rapidly (Callanan *et al.* 1992, Beebe *et al.* 1994, Hartmann 2012). The asymptomatic phase is the second stage of FIV infection. In this phase the cat's humoral immune response reduces the plasma virus load, but fails to clear the infection (Addie *et al.* 2000, McDonnel *et al.* 2013). As the disease progresses, a steady decline in CD4+ T helper cells occurs and antiviral immunity declines. Within months to years, the plasma virus level increases throughout the infection and finally leads to an immune deficiency stage which is considered as the end stage of the disease (Ackley *et al.* 1990, Hoffmann-fezer *et al.* 1992). Diagnostic investigations are usually based on antibody detection by enzyme-linked immunosorbent assay (ELISA), and sometimes on virus detection by polymerase chain reaction (PCR) or on other tests detecting antigen (Bienzle *et al.* 2004, Hartmann *et al.* 2007). Proviral DNA quantification can provide useful information about disease staging, independent of the CD4+ count and can be detected by using the real-time PCR method (Shiramizu *et al.* 2005, Gueudin *et al.* 2008, Malnati *et al.* 2008). Cycle threshold (Ct) values are proportional to the amount of proviral DNA

in samples and correlate to both free in fluid and cell-associated proviral load. Therefore, it can be used for interpretation of FIV proviral levels (Désiré *et al.* 2001, Luo *et al.* 2005, Malnati *et al.* 2008).

Since biting and direct inoculation of infected blood and saliva are the main routes for transmission of FIV infection, intact male cats, which are more likely to display aggressive behavior following breeding and territorial fights, are usually more frequently infected (Winkler *et al.* 1999, Little 2005).

FIV and HIV are both neurotropic viruses, and neurological impairments are believed to be a consequence of direct viral effect (Pedersen and Barlough 1991, Meeker and Hudson 2017). In experimentally infected cats, behavioral disturbances like extreme aggression following neurodegeneration are widely seen by using a sensitive behavioral recording. However, whether naturally FIV-infected cats have the neurological disease it is still controversial. Clinically relevant neurobehavioral impairments are rarely observed in these patients (English *et al.* 1994, Phillips *et al.* 1994, Meeker 2007). Close monitoring of the aggressive behavior in naturally infected cats during the course of the infection can contribute to a better understanding of the neurobehavioral aspect of FIV disease. Thus, the aim of the current study is to determine the possible interaction between aggressive behavior and FIV disease progression based on semi-quantitative viral load levels and health status in naturally FIV-infected cats.

Materials and methods

Subjects and sampling

The study was conducted at the veterinary hospital of University of Tabriz from September 2015 to August 2016. Owned and stray cats older than 1-year were recruited because it was believed that current aggression tendencies in cats younger than 1-year may not be reflective of their aggression tendencies at adulthood since aggression in these cats could be related to their motivation of play in some situations. In the current study, most of the owned cats were recruited during the annual health check or during hospitalization for further workup of systemic illnesses. Stray cats instead were rescued by animal welfare groups and brought to the hospital for a diagnostic checkup before entering shelters. All recruited cats were clinically examined by board-certified small animal internal medicine specialists and each subject's health status was determined afterward. Cats were excluded from the study if:

- they had a known FIV infection status (due to prior testing);
- they had conditions that could cause extreme pain like traumatic injuries.

General health assessment was conducted after history taking by performing a routine physical and neurological examination and evaluating the complete blood count and biochemistry profile of the subjects. For determination of the health status, cats with at least one clinical abnormality related to common diseases and opportunistic infections following the terminal stage of FIV disease were considered as unhealthy (Ishida and Tomoda 1990, Bęczkowski *et al.* 2015). Diseases considered to be associated with FIV disease progression to the terminal stage included: chronic stomatitis/gingivitis with gingival score of 2/3 or 3/3 (signs: ptyalism, halitosis, and bleeding gingiva), chronic upper respiratory tract infections (signs: nasal discharge, stertorous breathing, ocular signs like conjunctivitis), chronic skin diseases e.g. parasitic, dermatophytosis or bacterial pyoderma (signs: pruritus, erythema, scaling, crusting, and alopecia).

Two to four ml of whole blood were collected from each cat after the clinical examination. Blood collections were done via standard venipuncture from the jugular vein in sterile microtubes with EDTA. Sedation with a dose of 0.05 mg kg^{-1} of acepromazine maleate 2% injected intramuscularly was done to sedate the cats with anxiety. Blood samples were submitted for proviral DNA extraction after isolation of the buffy coat layer via centrifugation (10,000 g, 6 minutes, 4 °C) and were stored at - 20 °C prior testing.

After the clinical procedure, as stray cats spent a quarantine period as long as their FIV status was determined. They were handled by a carrier into the cat's ward of the hospital and were kept in individually ventilated cages (1.5 x 2 meter in size). Commercial dry cat food was given to the cats twice a day. Wet cat diet was instead given to those cats which had gingivostomatitis. Owned cats were sent back to their home and shelter cats were sent to the shelters after completing the sample collection and clinical procedure.

During the quarantine period, the stray cats were taken out of the cage daily by a carrier and were brought to a room where they could interact with toys.

DNA extraction and PCR amplification of FIV

DNA was extracted from each blood sample with Genomic DNA purification kit (Bioneer, Korea) according to the instruction of the manufacturer. For the first stage reaction of FIV amplification, one pair of specific primers were used as described previously (Matteucci *et al.* 1993).

The sequence of the forward primer was 5'-GGCATATCCTATTCAAACAG-3'and the reverse primer was 5'-AAGAGTTGCATTTTATATCC-3'.

For the first stage reaction of the nested-PCR, 5 µl of each extracted DNA was used along with 1 µl of each primer, 12.5 µl of Master Mix (Ampliqon, Denmark) and 5.5 µl of nuclease-free water to reach the total volume of the reaction mixture into 25 µl. Thermal profile used for the first reaction included an initial denaturation at 94 °C for 3 min, followed by 40 cycles of 94 °C for 1 min for denaturation, 50 °C for 1 min for annealing, 72 °C for 120 sec for extension, and a final extension step at 72 °C for 5 minutes. Amplification products were 1/10 diluted with nuclease-free water in order to be submitted for the second reaction.

Real-time PCR as the second stage reaction

One pair of FIV specific primers were designed with Primer3 software and checked with OligoAnalyzer 3.1 software on FIV gene (NCBI access number: M25381.1) as following: the forward primer was 5'-TAATAATGGCCGCACCAGGG-3', and the reverse primer was 5'-TGCATCCTAGCTGGTGCAAA-3'. Five µl of the diluted PCR product was submitted to a real-time PCR along with 0.5 µl of each designed internal primer, 10 µl of SYBER green Master Mix for PCR (Takara, USA), and 4 µl of nuclease-free water to reach the total volume of the reaction mixture into 20 µl. Thermal profile used for the second stage reaction performed at 95 °C for 10 min as initial denaturation, then followed by 40 cycles of 95 °C for 10 sec and 45 sec at 60 °C. All reactions were performed in triplicates.

Finally, 5 µl of real-time PCR products were run for an electrophoresis with an 1% agarose gel containing DNA safe stain with 100 bp DNA ladder.

Evaluating the aggressive behavior in cats

It is quite common to create and use a survey based on opinions of the cats' guardians to evaluate the frequency and likelihood of aggressive behaviors in cats (Stelow *et al.* 2016). Assessment of aggression was also done based on cats' demeanor as determined by the owners or veterinarians (Bande *et al.* 2012).

Thus, aggression tendencies in the current study were evaluated based on the subject's behavioral background by considering a rating from a person familiar with the cat according to their knowledge of the cat's typical behavior. A short questionnaire was given to the owners at the time of the history taking and before determination of the cats' FIV status. The questionnaire included 3 questions concerning the cat's aggression tendencies toward strangers, family members, and other cats (Vapalahti *et al.* 2016, Ahola *et al.* 2017). Owners were asked to rate their cat's aggressive behavior at five levels from "not at all" to "very often" by considering all related intolerant behaviors ranging from hissing, spitting and growling to inflicting physical injuries (bite and claws). The questionnaire had to reflect the range of aggression tendencies from "extremely aggressive with humans and other animals" to "extremely accustomed to interacting with humans and other animals and very comfortable with them". Those owned cats which were reported to show intolerant behaviors toward all three categories (family, strangers, and other cats) at "often" and "very often" level, were rated as aggressive cats (cats with extreme aggression tendencies). Evaluation of the human-directed aggressive behavior in the stray cats was also measured for hiss, bite, and slap/scratch during the clinical procedure before sedation, the quarantine period inside the ward while being fed and also during the time they were handled to the playroom by the small animal medicine intern. The long-term behavioral history of the stray cats based on displaying inter-cat aggression with related intolerant behaviors was also collected following observation by a caretaker for 90 days in the shelters while he or she was unaware of the cat's FIV status. Only those feral cats which showed aggressive behavior during the clinical procedures, feeding, and handling to the playroom area and which were also reported to often show aggressive behaviors toward other cats during 90 days of observation inside the shelter, were considered to be in the aggressive cats' category.

Statistical analysis

Statistical analysis was performed in SPSS statistics version 24.0. Chi-square analysis was used to see whether FIV infection was more prevalent among the aggressive cats and to see if there is any significant association between health status of the FIV-infected cats and their aggression tendencies. Evaluating the difference in the mean Ct values between the aggressive and non-aggressive FIV-infected cats was done, using Student t-test. A one-way analysis of variance (ANOVA) was then used to test statistically significant differences in the mean Ct values between the four groups of FIV-infected cats including the healthy and unhealthy cats with and without extreme aggression tendencies, with Ct values serving as the dependent variable and the health status and aggression serving as independent variables. Data were presented as mean ± standard error of the mean (SEM) and the statistical difference was considered significant at $p < 0.05$.

Table I. *Clinical abnormalities detected during the health assessment in the unhealthy Feline immunodeficiency virus (FIV) positive and negative cats (n = 49).* The most frequent diseases detected in the unhealthy FIV-infected cats were feline chronic gingivostomatitis (FCGS) and URT diseases which were seen in 78% and 41% of the unhealthy FIV-positive cats, respectively.

Diseases associated with the terminal stage of FIV disease	Main signs/symptoms in patients	The most common sign of the disease among patients (comments)	Number of unhealthy cats with clinical abnormalities (%)		
			FIV-positive cats (n = 32)	FIV-negative cats (n = 17)	Total unhealthy cats (n = 49)
Feline chronic gingivostomatitis (FCGS)	Ptyalism, oral pain, halitosis, bleeding gingiva	Bleeding gingiva (appeared in all patients with FCGS during the clinical examination)	25 (78%)	4 (24%)	29 (59%)
Chronic upper respiratory tract (URT) infections	Nasal discharge, stertorous breathing, conjunctivitis	Stertorous breathing (more frequent with rhinitis in the FIV-positive patients)	13 (41%)	4 (24%)	17 (34%)
Chronic skin diseases (including bacterial, fungal, and parasitic infections)	Pruritus, erythema, scaling, crusting and alopecia	Scaling and crusting	7 (22%)	1 (6%)	8 (16%)

Results

Fifty-two owned and forty-four stray cats of different species and gender (32 females, 15 spayed females, 31 males, and 18 neutered males) were recruited for this study following inclusion and exclusion criteria. The median age of the whole study population was 3.48 ± 1.88 years, ranging from 1 to 12 years. Overall, forty-seven cats (49%) were considered to be clinically healthy in general and forty-nine cats (51%) were grouped as unhealthy based on the health assessment records (considering those with diseases associated with the terminal stage of FIV disease). Details of FIV-positive and negative cats within each disease category were shown in Table I.

The first stage reaction amplified a 675 bp DNA fragment and the second stage reaction amplified a 199 bp DNA fragment for the positive samples. Ct values were also collected for the FIV-positive samples following real-time PCR amplifications.

Finally, forty-seven cats (49%) out of ninety-six subjects were diagnosed as FIV-infected using the nested-PCR method. After evaluating aggression tendencies in all subjects, twenty-four cats were considered to be aggressive (25%) and seventy-two cats (75%) were included in the non-aggressive category. All aggressive cats (100%) and twenty-three of the non-aggressive cats (31.9%) were FIV-positive. Results also showed that twenty cats out of twenty-four aggressive FIV-infected cats (83%) in the current study were unhealthy. The mean Ct value which is semi-quantitatively proportional to the amount of proviral DNA in peripheral blood was 14.78 ± 0.91 (ranging from 9.91 to 29.27) for the aggressive and 21.39 ± 1.61 (ranging from 11.39 to 30.52) for the non-aggressive FIV-infected cats. Statistical analysis revealed a significant association between FIV prevalence and aggressive behavior in cats, (p-value = 0.000) (Table II). A significant association was also observed between health status and aggression in the FIV-infected cats (p-value = 0.022) (Table III). There was also a significant difference in the mean Ct values between the aggressive and non-aggressive FIV-infected cats (p-value = 0.012) (Figure 1). One -way ANOVA revealed a significant interaction in Ct values between the four groups of FIV-infected cats (the healthy aggressive vs. healthy non-aggressive vs. unhealthy aggressive vs. unhealthy non-aggressive cats), indicating that changes within the Ct values between these

Table II. *Pattern of Feline immunodeficiency virus (FIV) status and aggression within the whole study population.* All aggressive cats were PCR-positive for FIV disease and there was a significant association between the prevalence of FIV infection and aggression tendencies in cats (p =0.000).

FIV status	With aggression	Without aggression	Total	X^2	P value
Positive	24	23	47	33.362	< 0.001
Negative	0	49	49		
Total	24	72	96		

Table III. *Pattern of Aggression and health status within the Feline immunodeficiency virus (FIV) positive cats.* Twenty cats (83%) out of the twenty-four aggressive FIV-positive cats were unhealthy. There was a significant association between the health status of FIV-infected cats and aggression tendencies (p = 0.022) which shows that the aggressive FIV-positive cats were more likely to have an unhealthy status compared to the non-aggressive cats.

Behavior status	Healthy	Unhealthy	Total	X^2	P value
Aggressive	4	20	24		
Non-aggressive	11	12	23	5.248	0.022
Total	15	32	47		

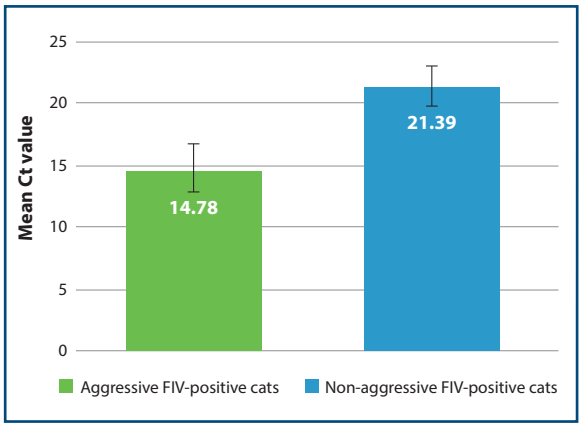

Figure 1. *Mean Ct values for Feline immunodeficiency virus (FIV) infected cats with and without aggressive behavior.* There was a significant difference in the mean Ct values between the aggressive and non-aggressive FIV-positive cats ($p = 0.012$) which shows that the aggressive FIV-positive cats significantly had a higher amount of proviral DNA in their peripheral blood compared to the non-aggressive cats and might be in a more progressed stage of the disease.

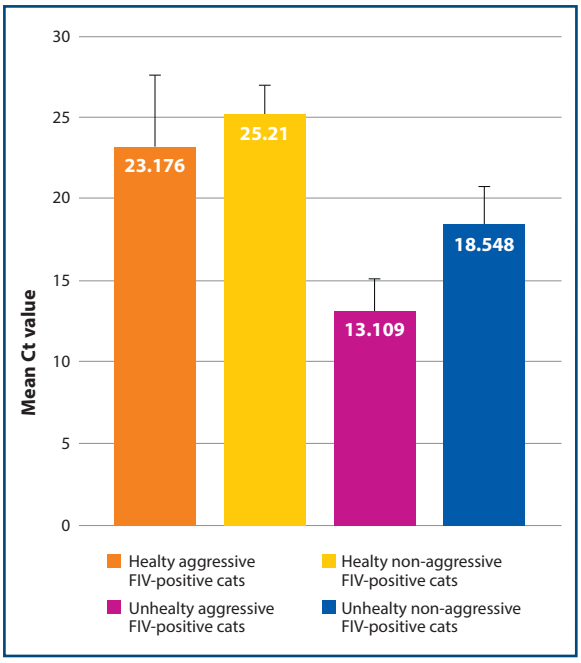

Figure 2. *The mean Ct values of the Feline immunodeficiency virus (FIV) positive cats grouped based on their aggression tendency and health status.* The *p*-value for the mean Ct values difference between the four groups was 0.001 showing that the difference between groups was significant. The post-hoc test revealed that the amount of proviral DNA based on the Ct values was significantly higher in the unhealthy aggressive cats compared to the healthy non-aggressive cats which shows a strong link between parameters associated with FIV disease progression and aggressive behavior.

groups were significantly different [F (3.43) = 6.099, *p*-value = 0.001] (Figure 2). The Games-Howell post-hoc test showed that the difference between the healthy group without extreme aggression tendencies (mean Ct value = 25.210 ± 1.74) and the unhealthy group with extreme aggression tendencies (mean Ct value = 13.109 ± 1.97) was significant ($p < 0.001$).

Discussion

Results of the current study showed that FIV disease is more prevalent in the aggressive cats and the aggressive FIV-infected cats were more likely to have an unhealthy status comparing to the non-aggressive FIV- infected individuals. Cats which displayed extreme aggression tendencies were all diagnosed as FIV-positive and they had a significantly lower Ct values and higher amount of proviral DNA than the non-aggressive FIV-infected cats. The statistically significant difference in Ct values between the four groups of healthy and unhealthy FIV- infected cats with and without extreme aggression tendencies ($p < 0.001$) also shows a direct link between the two parameters associated with FIV disease progression and aggressive behavior.

Gleich and colleagues (Gleich *et al.* 2009) reported a FIV prevalence rate of 91% in the aggressive cats in their study. In another study, the prevalence of FIV disease was also reported as 20.7% in the aggressive cats while the prevalence in the non-aggressive cats was much lower (9.6%) (Bande *et al.* 2012). Other studies also demonstrated that cats with more aggression tendencies have a greater risk for FIV infection (Yamamoto *et al.* 1989, Bradshaw and Hall 1999, Goldkamp *et al.* 2008, Lara *et al.* 2008, Gates and Dale 2017).

Ct values which have been shown to be semi-quantitative measures of the amount of virus in the clinical specimen can be obtained by real-time PCR assays, and if associated with factors related to disease severity like health status, may provide a reliable way for determining the progression of FIV disease (Bęczkowski *et al.* 2015). To date, the level of FIV proviral DNA among infected cats were evaluated in very few studies and all have revealed that as the disease progresses into the terminal stage, the amount of proviral DNA will increase as well (Klein *et al.* 1999, Pedersen *et al.* 2001, Ryan *et al.* 2003, Pinches *et al.* 2007, Leal *et al.* 2015).

The FIV-associated neurodegenerative disease is believed to cause a very gradual decline in the brain function with only a few overt signs until 5 to 8 years post-infection and it is also believed that a progressive encephalopathy usually overlaps with the development of acquired immune dysfunction in infected cats (Meeker and Hudson 2017). However, neurobehavioral disturbances still remain a controversial consequence of FIV disease progression in naturally infected cats. To our knowledge, no study has been evaluated

the interaction between a behavioral trait like aggression and variables associated with FIV disease progression so far. While the aggressive behavior has been already reported as a risk factor for FIV infection, our results may provide the very first link between aggression tendencies and parameters associated with FIV disease progression into the terminal stage. Aggression in cats is always considered as one of the important predisposing factors for FIV disease, and research studies have also reported a significant correlation between aggression and FIV infection (Fromont *et al.* 1997, Goldkamp *et al.* 2008, Gleich *et al.* 2009). Lara and colleagues (Lara *et al.* 2008) suggested that being aggressive as a risk factor for FIV infection is probably more important than the way of life in cats. On the other hand, as it was previously reported in a study conducted on the experimentally infected cats, exacerbated forms of aggression which can be a manifestation of social dysfunction may also be a consequence of the neurodegenerative effect caused by FIV (Dow *et al.* 1990, Meeker 2007). Thus, FIV may play a critical role in disrupting social-emotional behaviors like aggression in naturally infected cats similar to what has been documented in experimentally infected individuals and the progressive encephalopathy following FIV disease progression may exacerbate aggression tendencies in patients regardless of originally being a cat with extreme aggression tendencies. Podell and colleagues (Podell *et al.* 1997) also demonstrated that progressive encephalopathy parallels the reduction in the CD4/CD8 ratio among the experimentally infected FIV cats. Therefore, in regard to the previous studies, it can be indicated that FIV disease progression could influence the neurobehavioral function of the experimentally infected cats.

In this study FIV-infected cats with aggression tendencies had higher amounts of proviral DNA copies comparing to the healthy non-aggressive cats; thus, an assumption can be raised about the possible occurrence of neurodegeneration following the direct effect of FIV in naturally FIV-infected cats. It could be stated that the disease progression may possibly exacerbate aggression tendencies among the infected cats and consequently, increase the risk of FIV infection itself. However, the higher amount of proviral DNA in cats displaying extreme aggression tendencies can also be due to the greater risk they have for FIV infection. They might be involved in inter-cat fights when they were young and consequently, they have been infected earlier than non-aggressive cats. Hence, the aggressive FIV-infected cats in the current study may be in a more progressive stage of the disease at the time of the diagnosis and have a higher amount of proviral DNA than the non-aggressive FIV positive cats. That is why further studies are needed to confirm this hypothesis. There are also other factors that may have impacts on aggression tendencies in cats. For instance, being an outdoor cat can cause stress in different conditions which not only can cause mood and behavioral changes but also can affect the immune system and subsequently, cause a poor immune response to the virus replication. Current evidence also showed that dysregulation of the immune system may also be one of the mediators of social stress that causes exacerbation of aggression in both animals and humans (Takahashi *et al.* 2018); thus, disarray of the immune function following FIV disease progression may cause behavioral disturbances per se and should be discussed in future studies.

It is also important to know whether cats were also aggressive before and aggressive behaviors become more apparent and frequent recently or not. Such a long-term behavioral history can be collected from owned cats but in terms of having also free-roaming or stray cats among the study population, this is actually a great challenge as the way these cats behave in shelters may vary with their behavioral characteristics in the outdoor environment. It is also possible that human-directed aggression among the stray cats was the result of the 'stress' they experienced during the experimental procedure or because of the limited exposure to humans they had before. That is why we also tried to evaluate inter-cat aggression tendencies in these cats via observation for 90 days inside the shelters. However, our measure of aggression tendencies may have some limitations; for example, the answers reported in each section of the questionnaire were the subjective opinions of the cats' owners or caretakers. It would have been better to have objective observations in order to get more reliable answers. Nonetheless, answers related to human-directed aggression were strongly in accordance with the observed behaviors during the veterinary visits.

Overall, results of the current study showed that unhealthy status and high semiquantitative levels of FIV proviral load were significantly linked to extreme aggression tendencies. However, the subtlety of the results from the current study suggests the need for additional research on the topic. Thus, findings should advisedly be confirmed in additional populations and further studies is also needed to support the assumption about the exacerbation of aggression tendencies in cats following the possible effect of FIV through the course of natural infection.

Acknowledgements

We would like to thank all cat owners, clinicians and veterinary staff in University of Tabriz veterinary hospital and central laboratory, animal shelters,

and welfare groups for their participation in this study. This study was conducted as a part of a thesis approved for the degree of Doctor of Veterinary Medicine (DVM) and partially funded by University of Tabriz postgraduate research department.

Statement of animal rights

Ethical approval was discussed and obtained (Approval code: FVM.REC.1396.940) by the ethics committee of Faculty of Veterinary Medicine, University of Tabriz in accordance with the national legislation. All cat owners and shelter groups were first informed about the objective of the study and those who agreed to participate in this study were then received a written informed consent to sign.

References

Ackley C.D., Yamamoto J.K., Levy N., Pedersen N.C. & Cooper M.D. 1990. Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus. *J Virol*, **64** (11), 5652-5655.

Addie D.D., Dennis J.M., Toth S., Callanan J.J., Reid S. & Jarrett O. 2000. Long-term impact on a closed household of pet cats of natural infection with feline coronavirus, feline leukemia virus and feline immunodeficiency virus. *Vet Rec*, **146** (15), 419-424.

Ahola M.K., Vapalahti K. & Lohi H. 2017. Early weaning increases aggression and stereotypic behaviour in cats. *Scientific Reports*, **7** (1), 10412.

Bande F., Arshad S.S., Hassan L., Zakaria Z., Sapian N.A., Rahman N.A. & Alazawy A. 2012. Prevalence and risk factors of feline leukaemia virus and feline immunodeficiency virus in peninsular Malaysia. *BMC Vet Res*, **8** (1), 33.

Bęczkowski P.M., Litster A., Lin T.L., Mellor D.J., Willett B.J. & Hosie M.J. 2015. Contrasting clinical outcomes in two cohorts of cats naturally infected with feline immunodeficiency virus (FIV). *Vet Microbiol*, **176** (1-2), 50-60.

Beebe A.M., Dua N., Faith T.G., Moore P.F., Pedersen N.C. & Dandekar S. 1994. Primary stage of feline immunodeficiency virus infection: viral dissemination and cellular targets. *J Virol*, **68** (5), 3080-3091.

Bienzle D., Reggeti F., Wen X., Little S., Hobson J. & Kruth S. 2004. The variability of serological and molecular diagnosis of feline immunodeficiency virus infection. *Canadian Vet J*, **45** (9), 753.

Bradshaw J.W. & Hall S.L. 1999. Affiliative behaviour of related and unrelated pairs of cats in catteries: a preliminary report. *Appl Animal Behaviour Sc*, **63** (3), 251-255.

Callanan J.J., Thompson H., Toth S.R., O'Neil B., Lawrence C.E., Willett B. & Jarrett O. 1992. Clinical and pathological findings in feline immunodeficiency virus experimental infection. *Vet Immunol Immunopathol*, **35** (1-2), 3-13.

Désiré N., Dehée A., Schneider V., Jacomet C., Goujon C., Girard P.-M. & Nicolas J.-C. 2001. Quantification of human immunodeficiency virus type 1 proviral load by a TaqMan real-time PCR Assay. *J Clin Microbiol*, **39** (4), 1303-1310.

Dow S.W., Poss M.L. & Hoover E.A. 1990. Feline immunodeficiency virus: a neurotropic lentivirus. *J Acquired Immune Deficiency Syndromes*, **3** (7), 658-668.

English R., Nelson P., Johnson C.M., Nasisse M., Tompkins W.A. & Tompkins M.B. 1994. Development of clinical disease in cats experimentally infected with feline immunodeficiency virus. *J Infect Dis*, **170** (3), 543-552.

Fromont E., Artois M., Langlais M., Courchamp F. & Pontier D. 1997. Modelling the feline leukemia virus (FeLV) in natural populations of cats (*Felis catus*). *Theoretical Population Biology*, **52** (1), 60-70.

Gates M.C., Vigeant S. & Dale A. 2017. Prevalence and risk factors for cats testing positive for feline immunodeficiency virus and feline leukaemia virus infection in cats entering an animal shelter in New Zealand. *New Zealand Vet J*, 1-7.

Gleich S.E., Krieger S. & Hartmann K. 2009. Prevalence of feline immunodeficiency virus and feline leukaemia virus among client-owned cats and risk factors for infection in Germany. *J Feline Med Surgery*, **11** (12), 985-992.

Goldkamp C.E., Levy J.K., Edinboro C.H. & Lachtara J.L. 2008. Seroprevalences of feline leukemia virus and feline immunodeficiency virus in cats with abscesses or bite wounds and rate of veterinarian compliance with current guidelines for retrovirus testing. *J Am Vet Med Ass*, **232** (8), 1152-1158.

Gueudin M., Damond F., Braun J., Taïeb, A., Lemée, V., Plantier J.C. & Simon F. 2008. Differences in proviral DNA load between HIV-1-and HIV-2-infected patients. *AIDS*, **22** (2), 211-215.

Hartmann K. 2012. Clinical aspects of feline retroviruses: a review. *Viruses*, **4** (11), 2684-2710.

Hartmann K., Griessmayr P., Schulz B., Greene C.E., Vidyashankar A.N., Jarrett O. & Egberink H.F. 2007. Quality of different in-clinic test systems for feline immunodeficiency virus and feline leukaemia virus infection. *J Feline Med Surgery*, **9** (6), 439-445.

Hoffmann-Fezer G., Thum J., Ackley C., Herbold M., Mysliwietz J., Thefeld S. & Kraft W. 1992. Decline in CD4+ cell numbers in cats with naturally acquired feline immunodeficiency virus infection. *J Virol*, **66** (3), 1484-1488.

Ishida T. & Tomoda I. 1990. Clinical staging of feline immunodeficiency virus infection. *Japanese J Vet Sci*, **52** (3), 645-648.

Klein D., Janda P., Steinborn R., Müller M., Salmons B. & Günzburg W.H. 1999. Proviral load determination of different feline immunodeficiency virus isolates using real-time polymerase chain reaction: influence of mismatches on quantification. *Electrophoresis*, **20** (2), 291-299.

Lara V.M., Taniwaki S.A. & Araújo Jr J.P. 2008. Occurrence of feline immunodeficiency virus infection in cats. *Ciência Rural*, **38** (8), 2245-2249.

Leal R.O., Gil S., Duarte A., McGahie D., Sepúlveda N., Niza M.M. & Tavares L. 2015. Evaluation of viremia, proviral load and cytokine profile in naturally feline immunodeficiency virus infected cats treated with two different protocols of recombinant feline interferon omega. *Res Vet Sci*, **99**, 87-95.

Little S.E. 2005. Feline immunodeficiency virus testing in stray, feral, and client-owned cats of Ottawa. *Can Vet J*, **46** (10), 898-901.

Luo W., Yang H., Rathbun K., Pau C.P. & Ou C.Y. 2005. Detection of human immunodeficiency virus type 1 DNA in dried blood spots by a duplex real-time PCR assay. *J Clin Microbiol*, **43** (4), 1851-1857.

Malnati M.S., Scarlatti G., Gatto F., Salvatori F., Cassina G., Rutigliano T. & Lusso P. 2008. A universal real-time PCR assay for the quantification of group-M HIV-1 proviral load. *Nature Protocols*, **3** (7), 1240-1248.

Matteucci D., Baldinotti F., Mazzetti P., Pistello M., Bandecchi P., Ghilarducci R. & Bendinelli M. 1993. Detection of feline immunodeficiency virus in saliva and plasma by cultivation and polymerase chain reaction. *J Clin Microbiol*, **31** (3), 494-501.

McDonnel S.J., Sparger E.E. & Murphy B.G. 2013. Feline immunodeficiency virus latency. *Retrovirology*, **10**, 69.

Meeker R.B. 2007. Feline immunodeficiency virus neuropathogenesis: from cats to calcium. *J Neuroimmune Pharmacol*, **2** (2), 154-170.

Meeker R.B. & Hudson L. 2017. Feline immunodeficiency virus neuropathogenesis: a model for HIV-induced CNS inflammation and neurodegeneration. *Vet Sci*, **4** (1), 14.

Miyazawa T., Tomonaga K., Kawaguchi Y. & Mikami T. 1994. The genome of feline immunodeficiency virus. *Archives of Virol*, **134** (3), 221-234.

Pedersen N.C. & Barlough J.E. 1991. Clinical overview of feline immunodeficiency virus. *JAVMA*, **199** (10), 1298.

Pedersen N.C., Ho E.W., Brown M.L. & Yamamoto J.K. 1987. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science*, **235**, 790-793.

Pedersen N.C., Leutenegger C.M., Woo J. & Higgins J. 2001. Virulence differences between two field isolates of feline immunodeficiency virus (FIV-APetaluma and FIV-CPGammar) in young adult specific pathogen free cats. *Vet Immunol Immunopathol*, **79** (1), 53-67.

Phillips T.R., Prospero-Garcia O., Puaoi D.L., Lerner D.L., Fox H.S., Olmsted R.A. & Elder J.H. 1994. Neurological abnormalities associated with feline immunodeficiency virus infection. *J General Virol*, **75** (5), 979-987.

Pinches M.D., Helps C.R., Gruffydd-Jones T.J., Egan K., Jarrett O. & Tasker S. 2007. Diagnosis of feline leukaemia virus infection by semi-quantitative real-time polymerase chain reaction. *J Feline Med Surg*, **9** (1), 8-13.

Podell M., HayesDagger K., OglesbeeDagger M. & MathesDagger L. 1997. Progressive encephalopathy associated with CD4/CD8 inversion in adult FIV-infected cats. *J Acquired Immune Deficiency Syndromes*, **15** (5), 332-340.

Ryan G., Klein D., Knapp E., Hosie M.J., Grimes T., Mabruk M.J. & Callanan J.J. 2003. Dynamics of viral and proviral loads of feline immunodeficiency virus within the feline central nervous system during the acute phase following intravenous infection. *J Virol*, **77** (13), 7477-7485.

Shiramizu B., Gartner S., Williams A., Shikuma C., Ratto-Kim S., Watters M. & Valcour V. 2005. Circulating proviral HIV DNA and HIV-associated dementia. *AIDS* (London, England), **19** (1), 45.

Stelow E.A., Bain M.J. & Kass P.H. 2016. The relationship between coat color and aggressive behaviors in the domestic cat. *J Appl Anim Welfare Sci*, **19** (1), 1-15.

Takahashi A., Flanigan M.E., McEwen B.S. & Russo S.J. 2018. Aggression, social stress, and the immune system in humans and animal models. *Frontiers Behavioral Neuroscience*, **12**, 56.

Vapalahti K., Virtala A.M., Joensuu T.A., Tiira K., Tähtinen J. & Lohi H. 2016. Health and behavioral survey of over 8000 Finnish cats. *Frontiers Vet Sci*, **3**, 70.

Winkler I.G., Löchelt M. & Flower R.L.P. 1999. Epidemiology of feline foamy virus and feline immunodeficiency virus infections in domestic and feral cats: a seroepidemiological study. *J Clin Microbiol*, **37** (9), 2848-2851.

Yamamoto J.K., Hansen H., Ho E.W., Morishita T.Y., Okuda T., Sawa T.R. & Pedersen N.C. 1989. Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission. *JAVMA*, **194** (2), 213-220.