# Prevalence of Feline Calicivirus in cats with Chronic Gingivitis Stomatitis

## and potential risk factors

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# Contents

# Abstract

Feline chronic gingivitis stomatitis (FCGS) is an inflammatory disease of the gingiva, oral mucosa and the pharynx in cats. There have been many speculations about the cause of this disease, including bacterial, viral and immunologic causes, but most likely the aetiology is multi factorial. Since the Feline Calicivirus (FCV) probably plays a causative role, this study investigated the prevalence of FCV in cats with FCGS. Three groups were formed, namely a group of 44 cats with FCGS, a control group of 49 cats and a group of 16 cats with other dental problems (i.e. periodontitis, ankylosis and/or replacement resorption). From each cat the presence of FCV and Feline Herpesvvirus-1 (FHV) was established by viral isolation from oropharyngeal swabs and information about living conditions and clinical history were obtained.

The prevalence of FCV in the FCGS group and in the group of cats with other dental problems was 95.5% and 37.5% respectively, against 4.1% in the control group. The prevalence of FHV was however very low in all three groups (0-6.3%).

In total 14 potential risk factors were analysed in an uni- and multivariable analysis. Positive statistical significant ($P \leq 0.05$) associations with, i.e. risk factors for FCGS, were the male sex (OR=4.1), purebreds (OR=25.2), and visitation at the pet shelter (OR=9.4). In addition, cats in the age category of <1 year were less likely to have FCGS (OR=0.031) compared to cats older than 12 years. All cats with a history of acute oral respiratory disease (AORD) had FCGS (100%). Purebreds are also predisposed for other dental problems. Risk factors for carrying FCV were; other cats in the household (OR=6.9), visitation at the pet shelter (OR=4.2) and a history of AORD (OR=126.1). In addition, cats in the age categories of 1-3 years (OR=14.4) and 3-6 years were more likely to shed FCV (OR=19.2) compared to cats over 12 years of age.

# Introduction

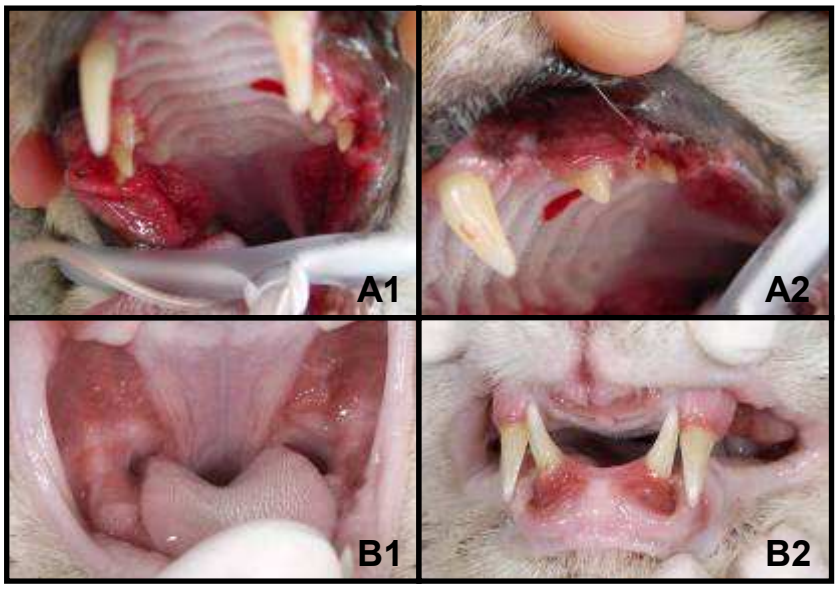
**Feline chronic gingivitis stomatitis**

Feline chronic gingivitis stomatitis (FCGS) is an inflammatory disease of the gingiva, oral mucosa and the pharynx in cats. Although this is described as a commonly occurring disease, the prevalence found in the literature is low [1, 2]. The disease is characterized by erythema, swelling, increased friability, bleeding, proliferations and ulcerations of the mucous membranes. Affected tissues are the gingiva, the oral mucosa, the glossopalatine arch (fauces) and the adjacent tissues [2-4] (Picture 1). In severe cases proliferative and ulcerative changes can also occur on the cheeks, tongue and lips [3, 4]. Affected cats can also develop a periodontitis [3]. FCGS occurs in cats of all ages, but is most frequently seen in adult cats [1, 4].

Purebreds tend to be predisposed in developing oral diseases in general [3, 5, 6], but in the case of FCGS a percentage of purebreds of about 10% ranging to 25% was found [1, 2, 4, 7].

Clinical signs most frequently seen with FCGS are difficulty with eating or even anorexia, halitosis, ptyalism, weight loss, and (in severe cases) even dehydration [2-4].

Histological examination of the damaged tissues shows a diffuse and dense cell infiltration in the mucosa and submucosa, containing plasma cells and lymphocytes [2, 4], an image which is to be expected with a chronic infection [6]. Hence, this inflammatory disease is also called *plasma cell gingivitis(-stomatitis)-pharyngitis* or *lymphoplasmacytic gingivitis.*




***Picture 1.*** *Two cats showing signs of FCGS.*
**A1-2**: Erythema, swelling, increased friability, bleeding and proliferations of the gingiva, fauces and adjacent tissues.
**B1-2**: Erythema of the gingiva, fauces and adjacent tissues (picture taken 3 months after full mouth extraction).

**Aetiology**

The cause of this inflammatory disease in cats is not clear. Most likely the aetiology is multi factorial. There have been many speculations about the causative factors that are responsible for this disease, as described below.

Immunity

When the balance between the host defences and the infection pressure is disturbed diseases can develop. A deficient local or systemic immune system can give infective agents the opportunity to colonize and invade the oral tissues and therefore immunodeficiency in general is a predisposing factor for developing FCGS [6, 8].

The first defences of the host immunity are the oral epithelium, the normal bacterial flora and the saliva that contains immunoglobulins, enzymes and leukocytes. In case these defences are overcome by infective agents the active immunity is triggered. The permeability of the capillaries increases (redness, swelling), inflammatory mediators are released (dolor), leukocytes migrate through the capillary walls and macrophages consume the invaded materials. The differentiation of lymphocytes to T lymphocytes and B plasma cells is started and subsequently immunoglobulins are produced by the B plasma cells [6, 8]. Especially IgG, IgM and IgA are produced, since the serum concentrations of IgG, IgM and IgA, and the saliva concentrations of IgG and IgM are increased in cats with FCGS [9]. These immunoglobulins are mainly responsible for the neutralisation (IgG, IgA) and opsonisation (IgG) of pathogens and the activation of the complement system (IgM) [10]. Strangely the concentration of IgA in the saliva is decreased, by which the immune response is missing a part of its ability to neutralize pathogens [9].

A deficient immune response, but also an over active immune system can cause chronic inflammation. Certain pathways of the host immunity, like hypersensitivity, polyreactivity and autoimmunity can lead to host tissue destruction and chronic inflammation of the oral tissues [6].

Bacteria

Bacterial infections can also play a role. Especially gram-negative anaerobe bacteria have been pointed out, since these bacteria are more frequently found in cats with FCGS than in cats without FCGS [3, 11]. The celmembrane of gram negative anaerobe bacteria contains LPS and this plays an important role in the initiation of periodontitis [12]. Moreover, gram-negative anaerobe bacteria are also an important aetiopathologic factor in oral infections in humans [11]. Full mouth extractions can lighten and even remove the inflammation [13, 14]. This suggests that dental plaque and calculus with all their residential bacteria play an important role in maintaining the inflammatory oral condition, however, antibiotics are often not curative [4, 13, 15, 16]. Thus it is unlikely that bacteria are a primary cause [3].

Viruses

Viruses have also been suggested as causes for FCGS, either direct or indirect. Feline Immunodeficiënty Virus (FIV), Feline Herpesvirus-1 (FHV), Feline Leukemia Virus, and Feline Calicivirus (FCV) have been investigated [1, 2, 5, 15-21]. All these viruses are shed in the saliva therefore it is most likely that these viruses contribute to or cause oral diseases.

Tenorio et al. (1990) investigated the prevalence of oral lesions in cats that were chronically infected with FIV, FeLV and/or FCV [5]. This study showed that the presence of oral lesions and the severity of these lesions were greater in cats with FIV than in cats without FIV. Moreover FIV infected cats co-infected with either FeLV or FCV showed even more oral lesions with greater severity than cats with only FIV [5, 21]. Another study reported that the prevalence of FIV is significantly higher in cats with FCGS than compared with the control group, 81% versus 16% respectively [17].

Although FHV is often seen in a co-infection with FCV, it is not clear whether FHV alone would cause FCGS. Contradictory studies [18, 20] have been published about the role of FHV

in FCGS; one reported a high prevalence (92%) but this was also in combination with a FCV infection [18] and another reported a prevalence of 0% [20].
On the other hand several studies have been published about the importance of FCV in the aetiology of FCGS [1, 17-20]. Prevalence of FCV in cats with FCGS was found to be 79-92%, where only about 20% of cats without FCGS were FCV positive [17, 18].
Two case reports [15, 16] showed that cats tested negative for FCV after the stomatitis-gingivitis was cured, whereas they were tested positive for FCV before treatment. This indicates for a strong relationship between FCV and FCGS.
Although FeLV has also been suggested to be of causative importance, studies have not been able to confirm this hypothesis [2, 17, 19].

**Treatment**
FCGS is often not easy to cure. The following treatments are most often applied.

Thorough dental management and homecare
The first step is to make sure that all the dental elements are clear of plaque and calculus, supra- as well as subgingival. Periodontal affected teeth must be extracted or surgical corrected. The aim of this treatment is to remove the bacteria along with the plaque and calculus and by doing so reducing the stimulation of the inflammation [14, 22]. To retain this reduced infection pressure it is advisable to repeat this procedure every 4-12 months in combination with home care.
The biggest challenge in this treatment option is home care (i.e., keeping the teeth free from dental plaque). Brushing the teeth by the owner, has been proven to be an effective method but this requires a lot of dedication of the owner and is often not completely beneficial [3, 14].
Antibiotics
Systemic antibiotics can be administered alongside the dental treatment or when dental management appears to be non effective. The following antibiotics can be administered for a 14 to 21 day's period; metronidazole, clindamycin, enrofloxin, ampicillin, amoxicillin-clavulanic acid, lincomycin, spiramycin and tetracycline [3, 14].
Corticosteroids
Corticosteroids can also reduce the oral inflammation. These should be given with precaution, because the immune system can already be suppressed, hence this is one of the predisposing factors of developing FCGS in the first place [14].
Full mouth extractions
If all of the above is not successful, full mouth extractions can be performed. The aim of this treatment is to totally remove the teeth in order to prevent dental plaque accumulation. At first extraction of all premolars and molars is recommended, later on extraction of the canines and incisors can be considered. Cats manage very well without their teeth and in general do not have any problems with eating or drinking [7, 14, 22].
In a study about the effectiveness of this treatment 60% of the cats were completely cured of clinical disease, 20% showed significant improvement in which only plaque control was still required, 13% showed little improvement in which medical management was still necessary, and only 7% showed no improvement at all [7].

There are many other drugs described in the literature, like goldsalts, cyclosporine, megestrol acetate, interferon omega, thalidomide, levamisole and azathioprine [3, 4, 15, 16, 22]. Most of these drugs are still in the experimental phase and should only be used when the veterinarian has experience using these kinds of drugs.
Despite successful treated cats there are also still cats that will not completely respond to any of the treatments mentioned above.

**Feline Calicivirus**

The acute manifestation of a FCV infection is inflammation of the upper respiratory tract, in which nasal discharge and ocular discharge (due to conjunctivitis) are most frequently seen. Often cats also show oral lesions, fever, anorexia, lethargy, sneezing and stiffness (limping syndrome). In severe cases with more virulent strains of FCV even pneumonia can develop [23, 24]. Especially kittens get infected [20] and can remain persistently infected after recovery [25, 26].

Recently new highly virulent strains of FCV have emerged, resulting in systemic disease with high mortality rates (virulent systemic FCV (VS-FCV)). Besides respiratory tract disease VS-FCV can also cause cutaneous oedema, ulcerative dermatitis, anorexia and necrosis of internal organs like the liver, spleen and pancreas. So far outbreaks of VS-FCV have mostly occurred in the United Kingdom (UK) and the United States of America (USA). Strains from the distinct outbreaks were genetically different, so it seems like they emerged independent from each other [24].

FCV replicates and remains in the tonsillar epithelium and the adjacent fossa mucosa and is shed in the saliva [27, 28]. This can continue over a period of time after clinical infection [25, 26]. Therefore even when the clinical signs are gone, cats can still remain a source of infection for susceptible animals.

Coyne et al. (2006) studied the prevalence and the viral shedding patterns of FCV in naturally infected colonies of domestic cats. They revealed 3 different patterns of viral shedding; consistent shedders (27%) (≥75% FCV positive), intermittent shedders (51%) and non-shedders (22%) (0% FCV positive). This suggests that cats can be resistant to infection despite being continually exposed to the virus. And in spite of most of them being persistently infected all cats were healthy, apart from a few that showed some upper respiratory signs and chronic gingivitis. Cats older than 3 years were more likely to shed the virus than cats of 3 years and younger [25]. In contrast, some studies were not able to create long-term FCV shedders by oronasal inoculation of FCV strains. The period of shedding varied from 10 to 186 days post infection [27, 29-32]. Based on previous studies, Coyne et al. (2006) concluded that the decrease in viral shedding after infection was exponential and only a small proportion becomes long-term shedder [25].

Cats can be vaccinated against FCV in order to minimize or prevent clinical signs upon infection. Unfortunately vaccination does not prevent infection and it also does not prevent development of a carrier state after infection [20, 33, 34]. This is why FCV can still spread in and between cat populations in spite of most cats being vaccinated. Contradictory, Porter et all (2008), reported a slighter chance of vaccinated cats being FCV positive then non-vaccinated cats [35].

Vaccination can be administered by two ways; subcutaneously and oronasaly. Vaccination could even play a role in increasing disease in a population, especially the oronasal vaccine, since the vaccine is directly administered at the site of replication. Therefore oronasal vaccines tend to generate more vaccine reactions then subcutaneous vaccines and the chance of shedding the virus after vaccination seems also higher with oronasal vaccines. Most of the vaccinations are administered subcutaneously. Also after subcutaneous vaccination clinical signs may occur. This might happen if the vaccine virus is accidentally infecting mucous membranes e.g. if vaccine is spilled at the injection site or if there is a leakage of the injection spot and thereby infecting a cat through grooming [34].

Most of the vaccines consist of a life attenuated FCV F9 strain. There have been some speculations about whether the now commonly used vaccine strain F9 is still protective against most of the field strains. Recent studies showed that F9 is still generating antibodies against most of the field strains [34-37] or at least some of them [38]. One way to improve

vaccine protection is to combine 2 or more strains in the vaccine and/or use strains with a broad cross reactivity [38].
FCV belongs to the family of Caliciviridae and has a small single-stranded, positive-sense RNA genome [24]. There are multiple strains of FCV which can differ in virulence and antigenicity [36, 39, 40]. Whether these different strains are also responsible for distinct clinical manifestations is not clear. Only one study could present a slight difference of 1 or 2 antisera in neutralization patterns between isolates originating from cats with chronic stomatitis and isolates from healthy cats and cats with acute oral/respiratory signs [36]. Two other studies however could not find any correlation between isolates of several expressions of FCV infection and their reactivity to neutralizing antisera [37, 40].
During persistent infection the virus is capable of remaining in the tonsilar tissues in spite of the presence of the immune system. One explanation is that the virus can change during passage in the host's tissues. Johnson (1992) revealed an antigenic change in 5 of 9 cats between the inoculated parent FCV strain and the isolated progeny strains after 35-169 days of persistent infection [41]. Another study also showed that the isolates from chronic origin were different from acute origin isolates in their serological relationships, suggesting that antigenic changes occur during persistent infection under immunologic selection and resulting in a chronic strain that is different from the acute isolate [39].
Although there are antigenic differences between isolates, they also show sufficient overlap [24], so whether there are multiple biotypes, all causing distinct diseases, or whether they all belong to the same biotype and capable of causing different forms of the disease, is not clear. So far, inoculation of different strains of FCV, all originating from cats with distinct expressions of FCV infection (chronic stomatitis, limping syndrome and acute oral/respiratory disease) only caused transient signs of acute oral/respiratory disease [29-32, 39].

**Aim of the study**
This study will try to ratify the role of FCV in FCGS and determine the prevalence patterns of FCV in cats with FCGS in the Netherlands, since these are still not completely clear.
The prevalence of FHV is also included in this study because FHV detection is standard performed next to FCV detection at the Department of Infectious Diseases and Immunology at the Faculty of Veterinary Medicine (Utrecht University).

In this study prevalence patterns of FCV and FHV are determined in cats with FCGS, cats with other dental problems, and in a control group. In addition, possible risk factors for FCGS, other dental problems and the presence of FCV are evaluated.

# Materials and methods

**Animals**

FCGS Group

Samples from 44 domestic cats with FCGS were obtained from The University Clinic for Companion Animals in Utrecht in the period from 2004 to 2009. Inclusion criteria for the FCGS group were:

- Gingivitis
- Pharyngitis (i.e. inflammation of the oral fauces and adjacent tissues)
- Recurring course of clinical signs.

Control Group

Samples from the control group containing 49 healthy domestic cats were partly obtained from The University Clinic for Companion Animals in Utrecht (24 cats) and partly from 5 general practices (25 cats), 4 practices in Utrecht and 1 in Voorschoten. This was done in the months March and April 2009. The control group had to meet the following criteria:

- No inflammation of the gingiva, oral mucosa and pharynx.
- No clinical signs that could indicate for acute oral/respiratory disease (nasal- and ocular discharge)
- No suspicion of acute oral/respiratory disease in the last six months.
- No severe internal disease.

Dental Group

Samples from a third group were added; 16 cats that suffered from periodontitis, ankylosis and/or replacement resorption. These cats were also obtained from The University Clinic for Companion Animals in Utrecht in the period from 2004 to 2009.

**Samples**

From each cat we obtained an orophayngeal swab, in order to establish viral presence. A correct sample was taken by brushing the tip of the swab firmly against the mucosa lateral to the oral fauces. The swab was then stored at -20°C until further analyzes.

A questionnaire was filled out either by the owner or through telephone contact with the owner, to obtain the following data from the cats that were included in one of the above groups:

- Age.
- Sex.
- Breed.
- Whether or not castrated.
- Total number of cats in household.
- Whether or not the cat had the ability to go outside.
- Visitation at pet shelters and boarding catteries (further on in this report there will only be spoken of pet shelter, as a combined word for both accommodations).
- Vaccination status.
- Clinical history concerning respiratory tract, oral cavity and eyes.

**Viral isolation**

Viral isolation took place at the Department of Infectious Diseases and Immunology, Utrecht University, Faculty of Veterinary Medicine.

Cells

Viral isolation was done on Crandell Reese Feline Kidney (CRFK) cells, from the American Type Culture Collection (ATCC). They were grown in Dullbecco's Modified Eagle's Medium (DMEM) with 10% fetal calf serum (FCS) and penicillin/ streptomycin (p/s), 10.000 U/ml, at 37°C, 5% $CO_2$.

The monolayers of CRFK cells were transferred into 6-well clusters. First the cells were washed with Phosphate Buffered Saline without Ca and Mg (PBS-0) and trypsinised with trypsin to detach the cells. The cells were then resuspended in DMEM + 10%FCS + p/s. Subsequently this cell suspension was, after removing the cloths, transferred to the 6-well clusters, each containing 4 glass coverslips and stored overnight at 37°C, 5% $CO_2$.

Infection

The material of each swab was thoroughly squeezed into 2,5ml of DMEM + 2%FCS. Afterwards this was pushed through a filter to remove any bacteria and yeast. The 6-well clusters containing the seeded CRFK cells were washed with PBS 2-diethylaminoethyl (DEAE) dextran and then infected with 1ml of the swab solution. After an incubation period of 1 hour the cells were washed again with PBS Mg/Ca and 2,5ml of DMEM + 2% FCS was added before they were stored again overnight at 37°C, 5% $CO_2$. The following 2 days the wells were checked for the presence of cytopathologic effect (CPE) (Picture 2).

CPE

In case CPE was discovered in the wells, the virus had to be identified using an immuunperoxidase colouring. First, the supernatant (passage 1) was taken out the well, centrifuged (5 min. 1500 RPM) and stored in 2 ampoules of 1ml at -20°C (without the sediment). Then the cells were washed with PBS Mg/Ca. In order to fixate only the CPE-positive wells the 4 glass coverslips had to be removed out of the wells and transferred into a 24 well cluster. The cells were then fixated with Methanol 95% acetic acid 5% and stored for 10 min at -20°C. Subsequently the wells were washed three times with PBS-0 with intervals of 5 minutes and one time short with MilliQ.

The 4 glass coverslips with cells were taken out of the wells and incubated for one hour with 50µl of different kinds of antisera on a peace of parafilm at room temperature. Two coverclips were incubated with antiserum against FCV (Cat anti Calici), one coverclip with antiserum against FHV (Cat anti FHV) and one coverclip with serum from a Specific Pathogen Free cat (SPF cat: negative control). The sera were diluted at 1:160, 1:100 and 1:100, respectively.

The antisera originate from SPF cats that were inoculated with either FCV or FHV-1. Therefore the antisera are against the whole viral particle and not just against a particular antigen of the virus.

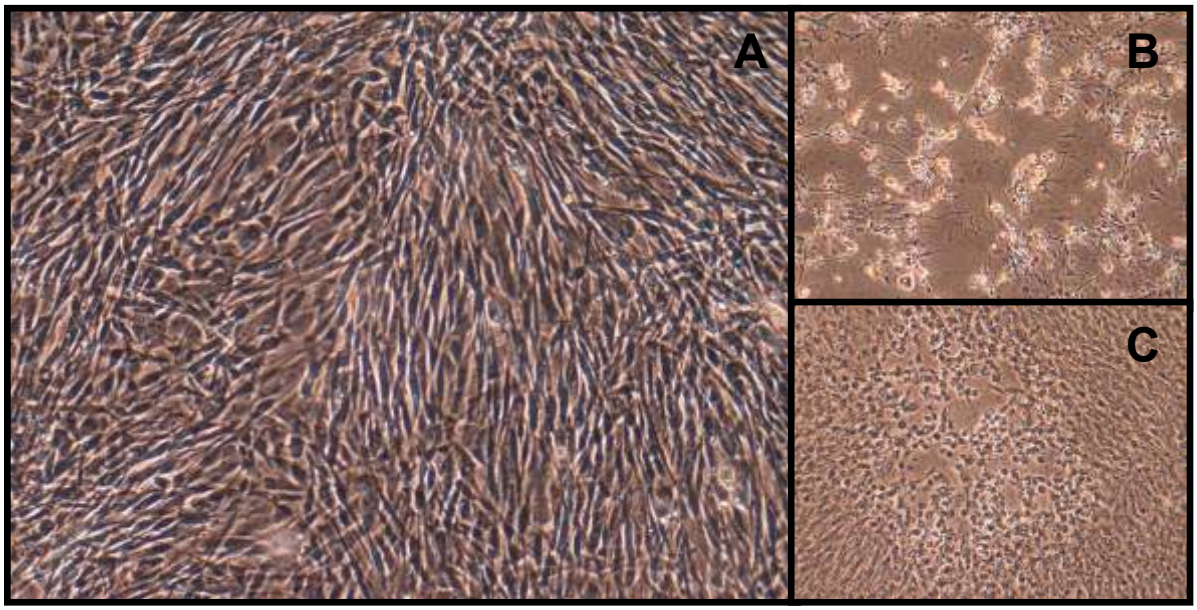
After incubation the coverclips were taken back into the 24 well cluster and washed three times with PBS-0 with intervals of 5 minutes. Then in each well 250µl of conjugate (Goat anti IgG cat FITC (Cappel)) was added, which was diluted at 1:150 and this was incubated for one hour in the dark at room temperature.

After incubation the wells were washed three times with PBS-0 with intervals of 5 minutes and one time short with MilliQ. Then the coverclips were laid down on a glass slide with a drop of 50% glycerol/50% MilliQ and viewed under an immunofluorescence microscope.

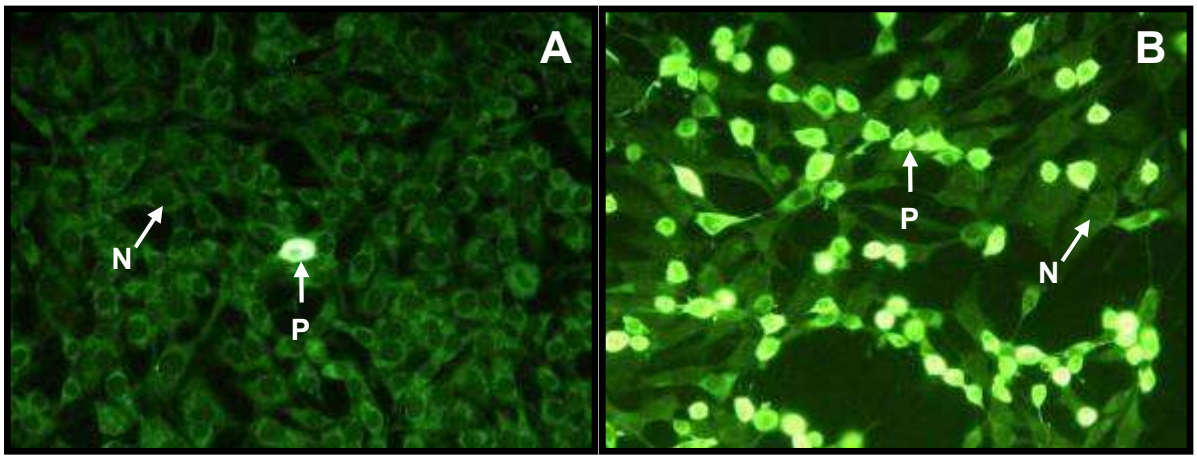
The samples were declared positive when the cells were fluorescent and negative when they were not (Picture 3).

No CPE

In case the wells did not show CPE within two days the cells had to be transferred into a bigger volume. The supernatant was taken from each well and placed into a TC-25 flask. Then the well was washed with PBS-0 and afterwards trypsinised. To completely detach the cells, the 4 coverclips had to be rubed with a plunger. The cells were then resuspended in 3ml of DMEM 5%FCS and added to the supernatant in the TC-25 flask. Flasks were then stored at 37°C, 5% $CO_2$. The following 3 days the flasks were checked again for CPE. In case the cells still did not show any CPE after this period, the samples were declared negative.




**Picture 2.** Microscopic image of CRFK cells. Monolayer without CPE (**A**), CFE of FCV (**B**) and CPE of FHV (**C**).




**Picture 3.** Immunofluorescence microscopic images of FCV positive (**P**) and FCV negative (**N**) CRFK cells.

**Statistical analysis**

Statistical analysis was performed using SPSS version 15.0. A database was formed and 2x*k* cross tables were made. Continuous variables for age, number of cats, last time vaccinated and the last time at the pet shelter were categorized. Comparisons were made between the FCGS group and control group, the dental group and the control group and between the FCV positive cats and the FCV negative cats. An univariable analysis was done using the Pearson $Chi^2$ test, in order to check if there was any association between the factors that were compared, i.e. if the $H_0$ hypothesis (there is no association between the factors) could be rejected. An association was statistically significant when the P value was less than or equal to 0.05 and then the nature of the association was established by calculating the Odds Ratio (OR) with a 95% confidence interval (CI) using the univariable logistic regression method, not taking any potential influential and confounding variables in account. The OR is a measurement for the association between the factors that are compared.

Calculation of the Odds Ratio:

**Odds of exposure of the diseased group (OED) =**
Fraction exposed in the diseased group / 1- fraction exposed in the diseased group
**Odds of exposure of the control group (OEC) =**
Fraction exposed in the control group / 1- fraction exposed in the control group

**The Odds Ratio** = (OED) / (OEC)

An OR of 1 means that there is no association between the disease and the potential risk factor, an OR of > 1 means that the exposed group has a greater risk of disease than the un-exposed and an OR of < 1 means that the exposed group has a smaller risk of disease than the un-exposed.

In order to take potential influential and confounding variables in account a multivariable logistic regression analysis was performed using the backward likelihood selection procedure. Only the variables with a P value of $\leq 0.25$ from the $Chi^2$ test were taken into the analysis. Variables that contained percentages of 0 and/or 100 (the 'unknown' categories excluded), and/or variables that contained a lot of missing values were unfortunately not compatible with the logistic regression method and were not taken into the multivariable analysis.

The 'unknown' categories were taken into all analyses, because without them there would be too many missing values. However test results of these categories are not shown because they are not interpretable.

# Results

**Age**

The mean age of the FCGS group, dental group and control group was 5.56 years (range 0.67-13.75), 7.78 years (range 0.66-16.00), and 4.90 years (range 0.17-17.42) respectively. The age distribution in categories for each group is shown in Figure 1.

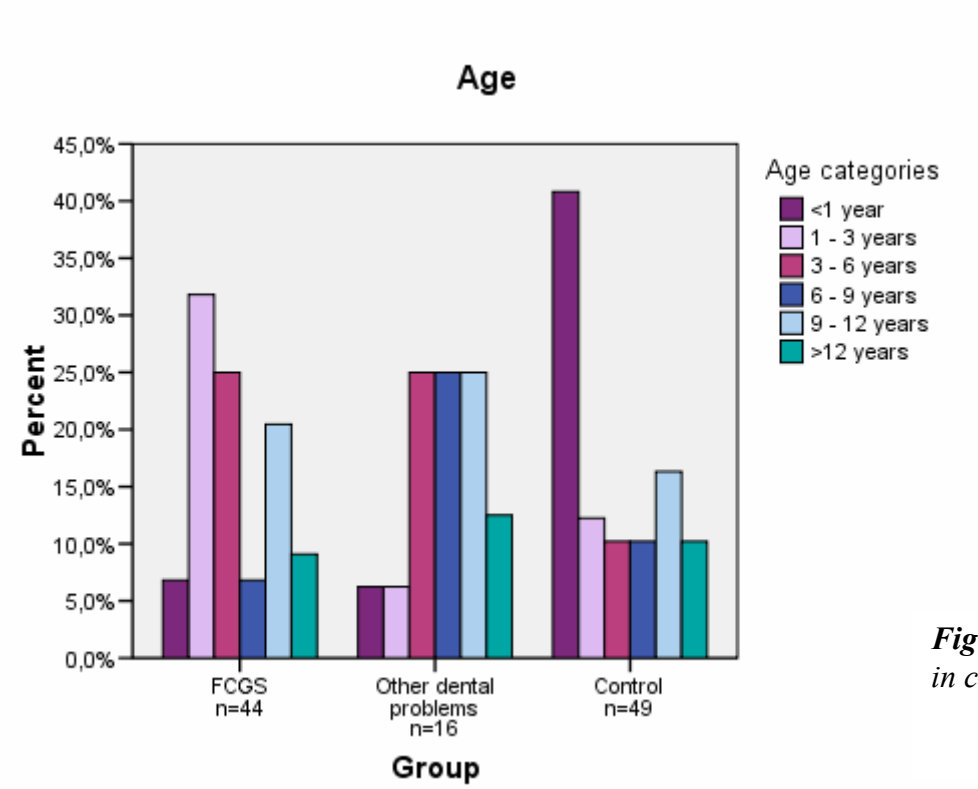



***Figure 1.*** *Age distribution in categories.*

**Sex**

The representation of males and females was 33/44 (75%) and 11/44 (25%) in the FCGS group, 9/16 (56.3%) and 7/16 (43.8%) in the dental group, and 20/49 (40.8%) and 29/49 (59.2%) in the control group respectively (Figure 2).
In the FCGS group 40/44 (90.9%) of the cats were castrated, in the dental group 15/16 (93.8%), and in the control group 36/49 (73.5%) (Figure 11 in appendix I).

**Breed**

In the FCGS group 21/44 (47.7%) of the cats were purebreds, 2/44 (4.5%) crossbreds, and 21/44 (47.7%) were categorized as domestic shorthair. For the dental group this was 6/16 (37.5%), 0/0 (0%), and 10/16 (62.5%) respectively, and for the control group this was 6/49 (12.2%), 1/49 (2.0%), and 42/49 (85.7%) respectively (Figure 3).
Thirteen of the 21 purebreds in the FCGS group (61.9%) were Main Coons, while in the other 2 groups there were only 2 and 0 Main Coons, respectively. The specific breed representations of the groups are shown in Figure 12 in appendix I.

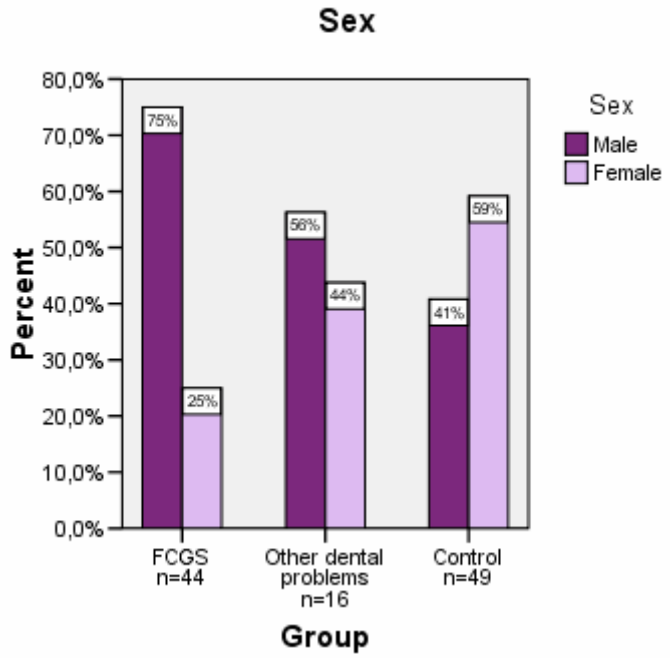



***Figure 2.*** *Sex distribution*

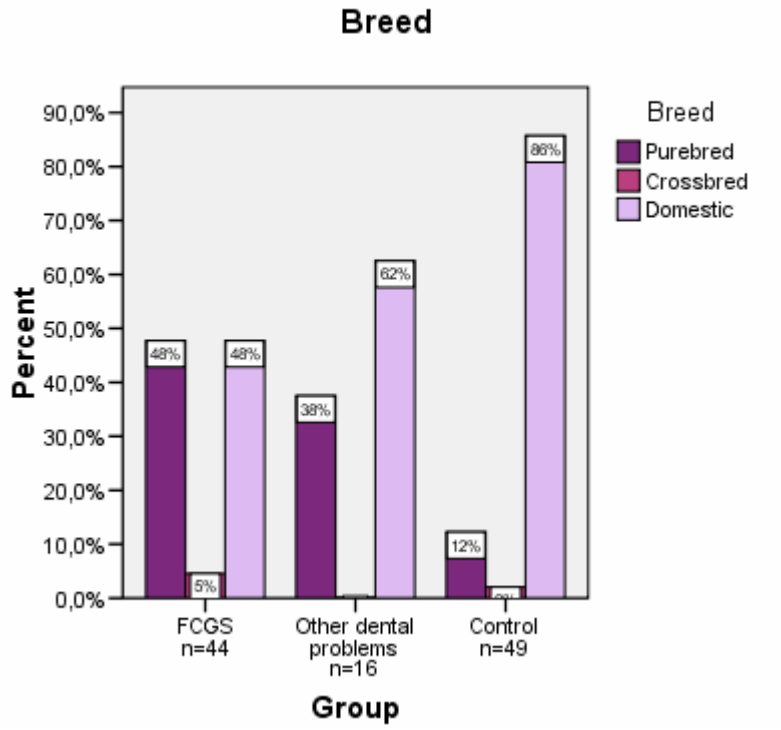



***Figure 3.*** *Breed distribution*

**Viruses**

Presence of FCV was established in 42/44 (95.5%) of the cats with FCGS. In the dental and control group this was 6/16 (37.5%) and 2/49 (4.1%) respectively (Figure 4).

FHV was detected in 1/44 (2.3%), 1/16 (6.3%), and 0/49 (0%) in the FCGS-, dental-, and control group respectively (Figure 5).

In 5 control cats the Feline Syncytial Virus (FeSV), also known as the feline foamy virus, was detected. The feline foamy virus can be widespread in cat populations. Transmission is most likely established through saliva by biting and licking. Microscopic examination reveals a characteristic foam-like effect (Picture 4), which is a result of syncytium formation,

cytoplasmic vacuolization and cell death. In spite of these cytopathic effects, the foamy virus seems to be harmless with no clinical signs while most cats stay life-long carriers [42, 43].

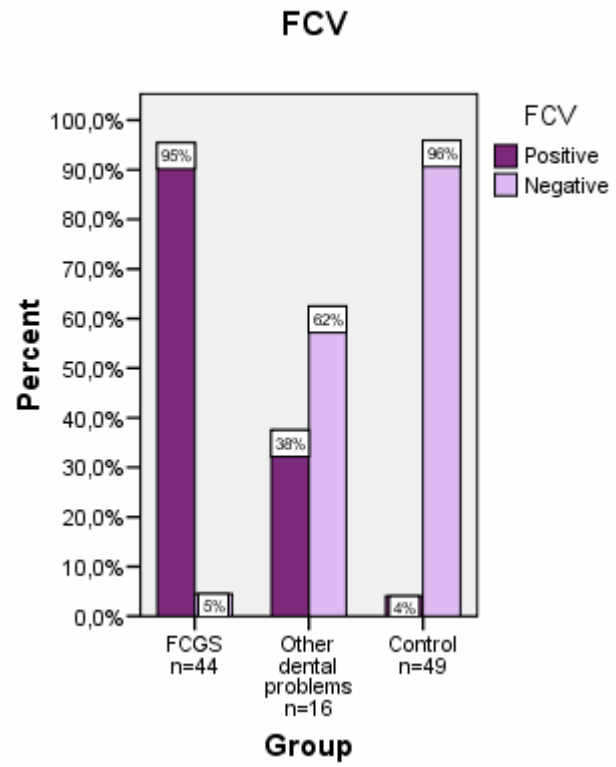



***Figure 4.*** *FCV prevalence*

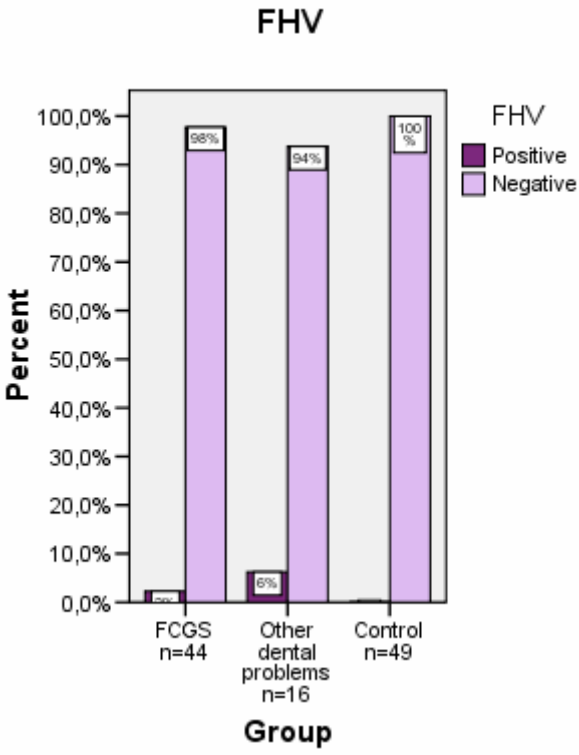
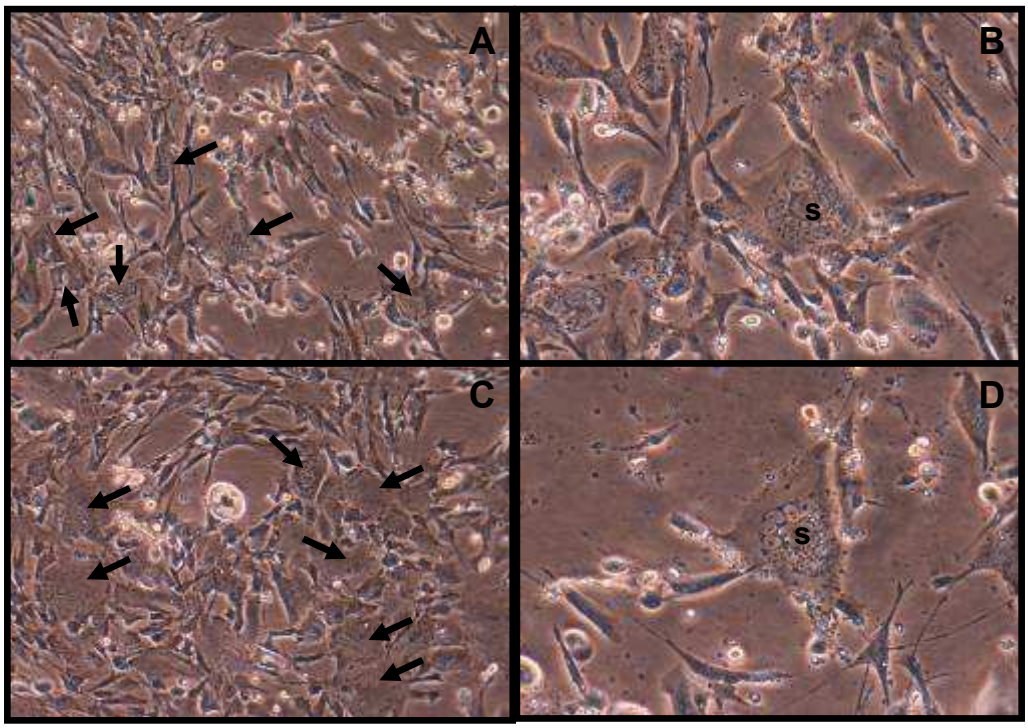



***Figure 5.*** *FHV prevalence*




***Picture 4.*** *Microscopic images of CRFK cells with FeSV, with the characteristic foamy aspect (**arrows**) and syncytium (**s**) forming.*

**Other cats in household**

Thirty-six of 44 (81.8%) cats in the FCGS group had other cats in the household. Of 4 (9.1%) cats this was unknown. In the dental group 10 of 12 (83.3%) cats had other cats in the household and of 4 (9.1%) cats this was unknown. In the control group this was 31 of all 49 (63.3%) cats (Figure 6). The number of other cats for each group is shown in Figure 13 in appendix I.

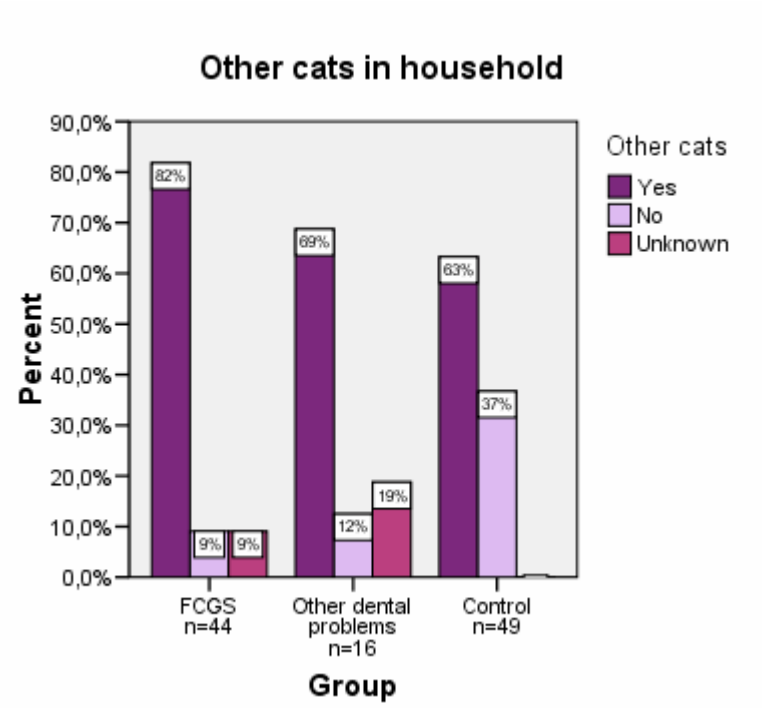



***Figure 6.*** *Percentages of cats with other cats in the household*

**Outside**

Twenty-three of 44 (52.3%) cats had the ability to go outside in the FCGS group and of 5 (11.4%) cats this was unknown. For the dental group this was 9 of 16 (56.3%) cats, of 6 (37.5%) cats this was unknown and for the control group 31 of 49 (63.3%) cats had the ability to go outside (Figure 7).

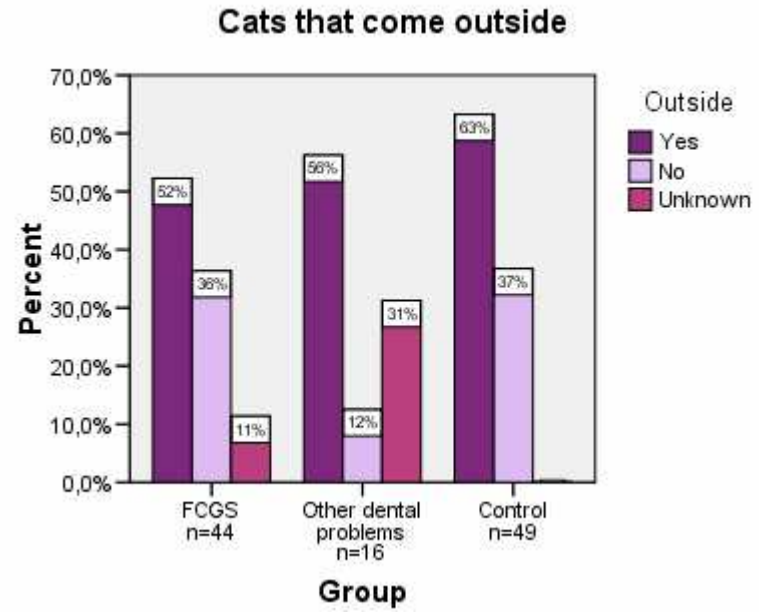



***Figure 7.*** *Percentages of cats that come outside*

**Vaccination**

In the FCGS group 37/44 (84.1%) of the cats were vaccinated for FCV and FHV and of 4 (9.1%) cats this was unknown. Eleven of 16 (68.8%) cats of the dental group were vaccinated, but of 5 (31.3%) cats this was unknown, and for the control group this was 39 of 49 (79.6%) cats with 2 (4.1%) missing data (Figure 8). For all vaccinated cats, the last time they were vaccinated is shown in Figure 14 in appendix I.

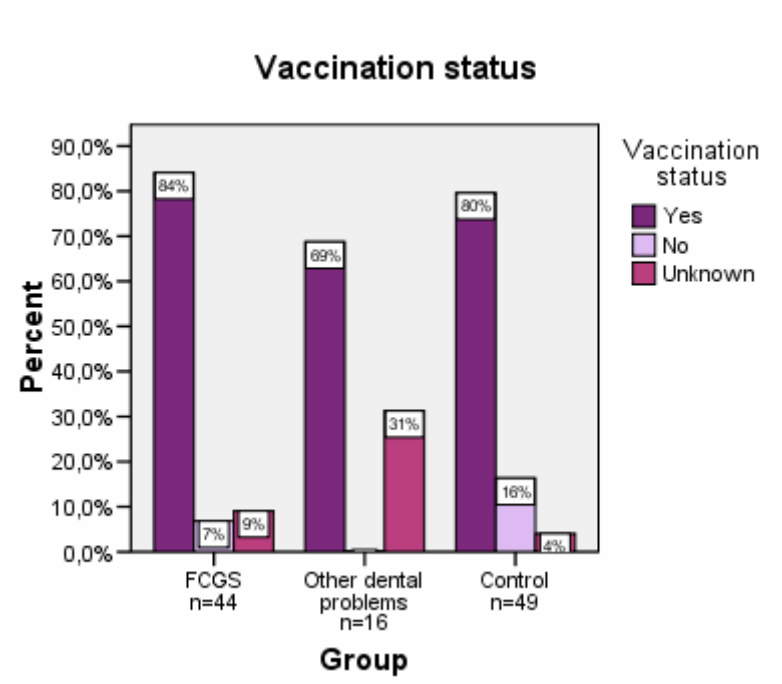



***Figure 8.*** *Percentages of cats that are vaccinated*

**Pet shelter**

Eighteen of 44 (40.9%) FCGS cats had visited a pet shelter and of 6 (13.6%) cats this was unknown. This was 1 of 16 (6.3%) in the group of cats with other dental problems, with 6 (37.5%) unknown data. Seven of 49 (14.3%) cats in the control group had visited a pet shelter, but of 1 cat (2.0%) this was unknown (Figure 9). The last time these cats have been to a pet shelter is shown in Figure 15 in appendix I.

**Clinical history**

Fifteen of 44 (34.1%) cats in the FCGS group have had signs of acute oral/respiratory disease (AORD) possibly caused by FCV and/or FHV and of 9 (20.5%) cats this was unknown. Signs of AORD had occurred in 2 of 16 (12.5%) cats in the dental group and in none of 49 (0%) cats with 6 (37.5%) and 2 (4.1%) unknown data respectively (Figure 10).

Of the 15 FCGS cats that have had AORD, 12 (80.0%) had other cats in the household, 8 (53.3%) had the ability to go outside, 2 (13.3%) were not vaccinated and 4 (26.7%) have been to a pet shelter.

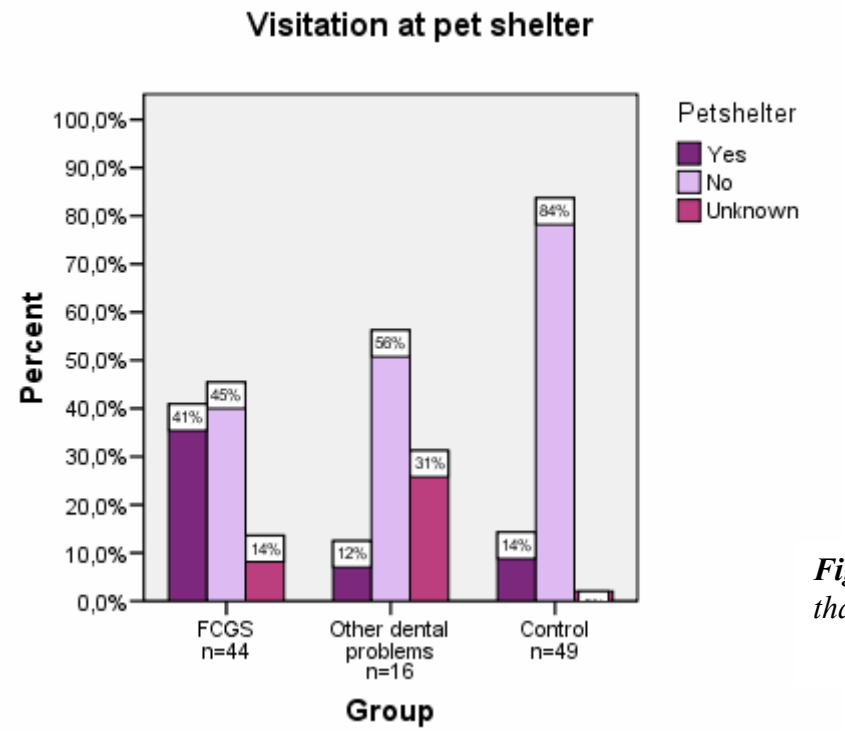



***Figure 9.*** *Percentages of cats that have been to a pet shelter*

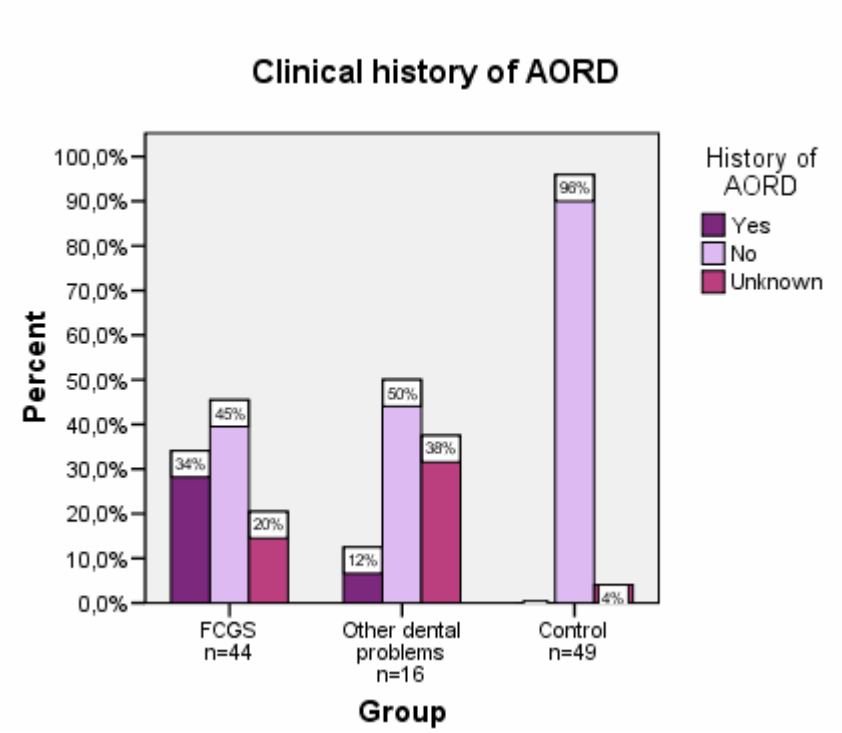



***Figure 10.*** *Percentages of cats with a history of AORD*

**Statistical analysis**
Detailed results of the statistical analyses are shown in table 4-9 in appendix II.

**FCGS vs Control**
Univariable analysis of possible risk factors
Comparing the FCGS group with the control group, using the $Chi^2$ test, 9 of the 14 variables showed a statistical significant ($P \leq 0.05$) association, including the presence of FCV ($Chi^2=77.6$; $P<0.001$), age ($Chi^2=18.5$; $P=0.002$), sex ($Chi^2=11.1$; $P=0.001$), breed ($Chi^2=15.4$; $P<0.001$), castration ($Chi^2=4.7$; $P=0.030$), other cats in the household ($Chi^2=13.1$; $P=0.001$), the ability to go outside ($Chi^2=6.1$; $P=0.049$), visitation at the pet shelter ($Chi^2=15.4$; $P<0.001$), and history of AORD ($Chi^2=30.2$; $P<0.001$) (Table 1).
Multivariable analysis of possible risk factors
After the potential influential and confounding factors were taken in account in the multivariable logistic regression, positive statistical significant ($P \leq 0.05$) associations with FCGS were found; the male sex (OR=4.1; CI95%=1.0-17.1), purebreds (OR=25.2; CI95%=2.7-235.7), and visitation at the pet shelter (OR=9.4; CI95%=1.8-50.0). A negative significant association was found with cats in the age category of <1 year (OR=0.031; CI95%=0.0-0.8) compared to cats older than 12 years (Table 1).
Included variables (with $P \leq 0.25$) in the multivariable analysis were age, sex, breed, castrated/intact, other cats in the household, the possibility to go outside and visitation at a pet shelter.
Feline calicivirus and a history of AORD could not be included. However FCV showed a positive association in the univariable analysis (OR=493.5; CI95%=66.5-3659.9) (Table 1).
Unfortunately no realistic Odds Ratio could be calculated for a history of AORD in the univariable analysis, because all of the cats who had a history of AORD also had FCGS (100%), but the percentages do point out a positive association.

**Dental vs Control**
Univariable analysis of possible risk factors
Comparing the cats with other dental problems with the control group, using the $Chi^2$ test, 7 of the 14 variables showed a statistical significant ($P \leq 0.05$) association, including the presence of FCV ($Chi^2=12.5$; $P<0.001$), other cats in the household ($Chi^2=11.5$; $P=0.003$), the ability to go outside ($Chi^2=17.7$; $P<0.001$), vaccination status ($Chi^2=11.1$; $P=0.004$), the last time the cats were vaccinated ($Chi^2=9.0$; $P=0.030$), visitation at the pet shelter ($Chi^2=12.4$; $P=0.002$), and a history of AORD ($Chi^2=20.1$; $P<0.001$) (Table 2).
Multivariable analysis of possible risk factors
For the multivariable analysis of the group with other dental problems a positive statistical significant association was only found with purebred cats (OR=18.7; CI95%=1.1-329.0) (Table 2).
Included variables (with $P \leq 0.25$) in the multivariable analysis were age, breed, castrated/intact, other cats in the household, vaccination status, the possibility to go outside and visitation at the pet shelter.
FCV, the last time the cats were vaccinated, and a history of AORD could not be included in the multivariable analysis. However FCV showed a positive association in the univariable analysis (OR=14.1; CI95%=2.5-80.3). The last time cats were vaccinated seemed statically significant in the $Chi^2$ test, but the univariable analysis does not confirm this (Table 2). For the same reasons a history of AORD could not be analysed.

**FCV**

Univariable analysis of possible risk factors

Comparing FCV positive cats with FCV negative cats, using the $Chi^2$ test, 7 of the 14 variables showed a statistical significant ($P \leq 0.05$) association, including clinical signs ($Chi^2=77.6$; $P<0.001$), age ($Chi^2=13.1$; $P=0.022$), sex ($Chi^2=8.6$; $P=0.003$), breed ($Chi^2=15.1$; $P=0.001$), other cats in the household ($Chi^2=8.8$; $P=0.012$), visitation at the pet shelter ($Chi^2=6.1$; $P=0.048$) and a history of AORD ($Chi^2=22.9$; $P<0.001$) (Table 3).

Multivariable analysis of possible risk factors

Positive statistical significant associations with the presence of FCV were; cats in the age category of 1-3 years (OR=14.4; CI95%=1.1-184.3) and 3-6 years (OR=19.2; CI95%=1.5-254.3) compared to cats over 12 years of age, other cats in the household (OR=6.9; CI95%=1.4-33.2), visitation at the pet shelter (OR=4.2; CI95%=1.1-16.5) and a history of AORD (OR=126.1; CI95%=7.2-2209.5) (Table 3).

Included variables (with $P \leq 0.25$) in the multivariable analysis were age, sex, breed, other cats in the household, the possibility to go outside, visitation at a pet shelter and a history of AORD.

Clinical signs could not be included in the multivariable analysis, but the OR's in the univariable analysis are the same as for the FCV prevalence in the two groups mentioned above (OR FCGS =493.5; CI95%=66.5-3659.9 and OR other dental problems =14.1; CI95%=2.5-80.3) (Table 3).

**Table 1.** Statistically significant (P≤0.05) associations between variables and the presence of FCGS.

| | | **Univariable analysis** | | **Multivariable analysis** | |
|---|---|---|---|---|---|
| Variable | FCGS freq. | OR | CI 95% | OR | CI 95% |
| **FCV** | | | | | |
| Positive | 42/44 (95.5%) | 493.500 | 66.544 – 3659.872 | † | |
| Negative (R) | 2/49 (4.1%) | 1 | | | |
| **Age** | | | | | |
| < 1 year | 3/23 (13.0%) | 0.188 | 0.031 – 1.122 | 0.031 | 0.001 – 0.872 |
| 1-3 years | 14/20 (70.0%) | 2.917 | 0.574 – 14.824 | | |
| 3-6 years | 11/16 (68.8%) | 2.750 | 0.509 – 14.860 | | |
| 6-9 years | 3/8 (37.5%) | 0.750 | 0.107 – 5.238 | | |
| 9-12 years | 9/17 (52.9%) | 1.406 | 0.277 – 7.131 | | |
| >12 years (R) | 4/9 (44.4%) | 1 | | 1 | |
| **Sex** | | | | | |
| Male | 33/53 (62.3%) | 4.350 | 1.788 – 10.581 | 4.134 | 0.997 – 17.135 |
| Female (R) | 11/40 (27.5%) | 1 | | 1 | |
| **Breed** | | | | | |
| Purebred | 21/27 (77.8%) | 7.000 | 2.455 – 19.957 | 25.244 | 2.704 – 235.671 |
| Crossbred | 2/3 (66.7%) | 4.000 | 0.343 – 46.676 | | |
| Domestic (R) | 21/63 (33.3%) | 1 | | 1 | |
| **Castrated/Intact** | | | | | |
| Yes | 40/76 (52.6%) | 3.611 | 1.079 – 12.082 | | |
| No (R) | 4/17 (23.5%) | 1 | | | |
| **Other cats in household** | | | | | |
| Yes | 36/67 (53.7%) | 5.226 | 1.598 – 17.093 | | |
| No (R) | 4/22 (18.2%) | 1 | | | |
| Unknown | 4/4 (100.0%) | | | | |
| **Outside** | | | | | |
| Yes | 23/54 (42.6%) | 0.835 | 0.352 – 1.978 | | |
| No (R) | 16/34 (47.1%) | 1 | | | |
| Unknown | 5/5 (100.0%) | | | | |
| **Pet shelter** | | | | | |
| Yes | 18/25 (72.0%) | 5.271 | 1.894 – 14.673 | 9.433 | 1.781 – 49.970 |
| No (R) | 20/61 (32.8%) | 1 | | | |
| Unknown | 6/7 (85.7%) | | | 1 | |
| **AORD** | | | | | |
| Yes | 15/15 (100.0%) | † | | † | |
| No (R) | 20/67 (29.9%) | 1 | | | |
| Unknown | 9/11 (81.8%) | | | | |

† Percentages not compatible for OR calculation
(R) Reference category

**Table 2.** Statistically significant (P≤0.05) associations between variables and the presence of other dental problems.

| | | **Univariable analysis** | | **Multivariable analysis** | |
|---|---|---|---|---|---|
| Variable | Frequency of other dental problems | OR | CI 95% | OR | CI 95% |
| **FCV** | | | | | |
| Positive | 6/8 (75.0%) | 14.100 | 2.475 – 80.315 | † | |
| Negative (R) | 10/57 (4.1%) | 1 | | | |
| **Breed** | | | | | |
| Purebred* | 6/12 (50.0%) | | | 18.705 | 1.064 – 328.938 |
| Crossbred | 0/1 (0.0%) | | | | |
| Domestic (R) | 10/52 (19.2%) | | | 1 | |
| **Other cats in household** | | | | | |
| Yes | 11/42 (53.7%) | 3.194 | 0.635 – 16.052 | | |
| No (R) | 2/20 (10.0%) | 1 | | | |
| Unknown | 3/3 (100.0%) | | | | |
| **Outside** | | | | | |
| Yes | 9/40 (22.5%) | 2.613 | 0.508 – 13.451 | | |
| No (R) | 2/20 (10.0%) | 1 | | | |
| Unknown | 5/5 (100.0%) | | | | |
| **Vaccination** | | | | | |
| Yes | 11/50 (22.7%) | † | | | |
| No (R) | 0/8 (0.0%) | 1 | | | |
| Unknown | 5/7 (71.4%) | | | | |
| **Last time vaccinated** | | | | | |
| In past 3 months | 5/11 (45.5%) | 5.833 | 0.525 – 64.823 | | |
| In past 6 months | 0/14 (0.0%) | † | | | |
| In past 12 months | 2/12 (16.7%) | 1.400 | 0.105 – 18.615 | | |
| > 12 months ago (R) | 1/8 (12.5%) | 1 | | | |
| **Petshelter** | | | | | |
| Yes | 2/9 (22.2%) | 1.302 | 0.231 – 7.336 | | |
| No (R) | 9/50 (18.0%) | 1 | | | |
| Unknown | 5/6 (83.3%) | | | | |
| **AORD** | | | | | |
| Yes | 2/2 (100.0%) | † | | † | |
| No (R) | 8/55 (14.5%) | 1 | | | |
| Unknown | 6/8 (75.0%) | | | | |

† Percentages not compatible for OR calculation
(R) Reference category

**Table 3.** Statistically significant (P≤0.05) associations between variables and the presence of FCV.

| | | **Univariable analysis** | | **Multivariable analysis** | |
|---|---|---|---|---|---|
| Variable | FCV frequency | OR | CI 95% | OR | CI 95% |
| **Clinical signs** | | | | † | |
| FCGS | 42/44 (95.5%) | 493.5 | 66.544 – 3659.872 | | |
| Other dental problems | 6/16 (37.5%) | 14.100 | 2.475 – 80.315 | | |
| None (control) (R) | 2/49 (4.1%) | 1 | | | |
| **Age** | | | | | |
| < 1 year | 6/24 (25.0%) | 0.889 | 0.176 – 4.478 | | |
| 1-3 years | 14/21 (66.7%) | 5.333 | 1.069 – 26.613 | 14.448 | 1.133 - 184.309 |
| 3-6 years | 13/20 (65.0%) | 4.952 | 0.986 – 24.875 | 19.246 | 1.457 - 254.250 |
| 6-9 years | 4/12 (33.3%) | 1.333 | 0.223 – 7.980 | | |
| 9-12 years | 10/21 (47.6%) | 2.424 | 0.500 – 11.761 | | |
| >12 years (R) | 3/11 (27.3%) | 1 | | 1 | |
| **Sex** | | | | | |
| Male | 36/62 (58.1%) | 3.264 | 1.462 – 7.287 | | |
| Female (R) | 14/47 (29.8%) | 1 | | | |
| **Breed** | | | | | |
| Purebred | 24/33 (72.7%) | 5.444 | 2.195 – 13.505 | | |
| Crossbred | 2/3 (66.7%) | 4.083 | 0.352 - 47.301 | | |
| Domestic (R) | 24/73 (32.9%) | 1 | | | |
| **Other cats in household** | | | | | |
| Yes | 40/78 (51.3%) | 4.000 | 1.358 – 11.785 | 6.895 | 1.431 - 33.230 |
| No (R) | 5/24 (20.8%) | 1 | | 1 | |
| Unknown | 5/7 (71.4%) | | | | |
| **Pet shelter** | | | | | |
| Yes | 17/27 (63.0%) | 2.877 | 1.147 – 7.214 | 4.207 | 1.073 - 16.490 |
| No (R) | 26/70 (37.1%) | 1 | | 1 | |
| Unknown | 7/12 (58.3%) | | | | |
| **AORD** | | | | | |
| Yes | 16/17 (94.1%) | 34.000 | 4.257 – 271.533 | 126.112 | 7.198 - 2209.527 |
| No (R) | 24/75 (32.0%) | 1 | | 1 | |
| Unknown | 10/17 (58.8%) | | | | |

† Percentages not compatible for OR calculation
(R) Reference category

# Discussion

**Group characteristics**

The mean age in this study in the FCGS group was 5.56 years which is lower than reported earlier by other studies who found a mean age of 7 to 8 years for cats with FCGS [1, 2, 4, 7]. The mean age for the control group was even lower (4.90 years), probably because many of the cats that were obtained from general practices were young cats and kittens, who came for a check up and/or vaccination. This could be the reason for the result that cats younger than 1 year appear to have a smaller chance for having FCGS than cats older than 12 years (OR=0.031), since 40.8% of the control cats were under the age of 1. This could also explain the higher percentage of non-castrated animals in this group compared to the other two groups (26.5% vs. 9.1-6.2%). The raw data suggest a higher FCGS risk for the age categories 1-3 years and 3-6 years, but this has not been statistically confirmed.

Surprisingly the sex distribution in the FCGS group was not 1:1, what would be expected on the basis of a normal population and what is also found in other reports [1, 2, 4]. In this study the female/male ratio was 1:3. Which makes the male sex significantly positive associated with FCGS (OR= 4.1), however the 95% confidence interval (0.997 – 17.135) lies close to unity. Perhaps male cats are more exposed to infectious diseases, which might play a role in developing FCGS, because in general they have a greater territory outside and are more aggressive towards other male cats.

About half of the FCGS cats were purebreds (47.7%) and this was also significantly associated with FCGS (OR=25.2), which is not in accordance with others who found a percentage of about 10% ranging to 25% among FCGS cats [1, 2, 4, 7]. It was also striking and not reported before that many of these purebreds with FCGS were Main Coons (61.9%). Perhaps a kind of hereditary factor in the Dutch breeding population of Main Coons could explain this phenomenon. However, so far there have not been any reports on the possibility of heredity of FCGS. Purebreds are also more likely to develop other dental problems (OR=18.7) which has been described in the literature before [3, 5, 6].

**Viral prevalence**

This study confirms the results established by many other studies about the high prevalence of FCV in cats with FCGS [1, 17-20]. With a percentage of 95.5% of FCV in the FCGS group against 4.5% in the control group and an OR of almost 500, there is a strong relationship between FCGS and the presence of FCV. Cats with other dental problems are also predisposed for carrying FCV, but with a lower OR (14.1) than cats with FCGS. Unfortunately this variable could not be included in the multivariable analysis for both groups, because the percentages were too close to 0 and 100.

The prevalence of FCV among cats with no clinical signs in this study was established at 4.1%. Only one Swedish study also reported a low prevalence of FCV among healthy cats (2.6%) [44]. However others studies found a prevalence of about 20% [17, 18, 45] and one of 10% [35]. These samples were conducted in the UK [17, 35, 45] and the USA [18] of which one contained a lot more cats from catteries with more than 3 cats (up to more than 50 cats) [45] and another used a control group of cats with mild to moderate periodontal disease [18]. This could explain the difference in prevalence compared to our study, however the Swedish sample was also taken from catteries instead of private households.

The prevalence of FHV was very low in all three groups (2.3% (FCGS group), 6.3% (dental group) and 0% (control group)). This is in accordance with other studies where the prevalence of FHV in FCGS and control cats was also very low (0-1%) [20, 35, 44, 45]. The intermittent

shedding pattern of FHV could play a mayor role, by which these prevalence's are most likely underestimated. Another reason could be that the presence of FCV can mask the presence of FHV in a co-infection, since the CPE for FCV develops must faster than for FHV. In order to overcome this problem a TaqMan probe-based, quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay could be used, which is more sensitive and specific than viral isolation from cell cultures [46, 47]. Another factor that should be taken into account for both FCV and FHV is that the swab samples of the control group in this study were conducted by at least 7 different people of which not all of them were practiced with the sampling method. In addition not all cats were sedated or cooperative. Therefore it is possible that FCV and FHV carriers were missed by non-correct sampling.
FeSV was detected in 5 of the control cats, however not in the other groups. FeSV is detected very late in the viral isolation process, i.e. the CPE appears only after the cells have not shown any CPE for about a week. Since the other two groups contained a lot more cases that showed CPE in the first couple of days a possible FeSV infection would not have been noticed.

Problems with entering the data in the uni- and multivariable analysis could have been overcome if all sample characteristics were represented in each group, which could have been established by forming matched control groups and/or taking bigger samples from the Dutch cat population. Unknown data are generally hard to overcome but in this case the vast majority was caused by the fact that the cat owners of the diseased cats had to be contacted by telephone, up to 5 years after the oropharyngeal swab was obtained, in order to get the information about their cat's living conditions and clinical history. Many of these owners were unattainable or too much time had gone by for them to remember the answers to the questions.

**Risk factors**
Visitation at the pet shelter in the past, independent of when, can be considered as a risk factor for FCGS, with an OR of 9.4. Hence visitation at a shelter is also a risk factor for carrying FCV (OR=4.2) it is likely that FCV is the link between FCGS and visitation at a pet shelter. Another explanation could be that another potential causative infectious agent for FCGS is transmitted between cats at the pet shelter or maybe the facility and accommodation conditions at the shelters play a role in making cats more susceptible for developing FCGS.
A history of AORD also seems to be an important risk factor, because all cats that had a history of AORD also had FCGS (100%). However it was not possible to include this variable in the uni- or multivariable analysis. AORD is often caused by FCV [23] and infected cats often stay consistent shedders of FCV [25, 26]. Either the high prevalence of cats with a history of AORD among the FCGS cats is a cause for the high prevalence of FCV in this study or more likely an acute infection of FCV (AORD) can give a higher risk for developing FCGS in the future, because FCV plays in fact a causative role. Perhaps an acute infection of FCV alters the local immune response in the oral cavity, which makes the cats more susceptible to chronic inflammation in the future or maybe the virus stays dormant in the tonsillar tissues and becomes virulent again when the immune system is depressed. Another possibility is that the initial FCV strain undergoes antigenic changes during tissue passage [39, 41] in order to avoid the host immune response and even changes in a chronic disease inducing strain. However, strains isolated from cats with FCGS also cause acute infection after experimental inoculation. If an acute infection of FCV does increase the risk of developing FCGS, perhaps one should investigate the possibilities of developing a vaccine against infection instead of only against the symptoms of FCV.
Risk factors for infection of FCV have been reported before [35, 45, 46], but with different results. An association with other cats in the household was found in this study with an OR of

6.9. Unfortunately there are no results for the separate categories of the number of cats, because there were too many missing values. However, another report showed an increase in the OR as the number of cats in the household increases [35]. Another study did not find an association with other cats in the household [45], which is strange since it is likely for other cats being a potential source of infection to other cats in the household. Unless non-infected cats can not come in contact with potential infected cats outside, the number of cats inside the household would not make a difference. For the same reason it is strange that the ability for cats to come outside is not a predisposing factor for FCV infection in this study, since the chance of infection would be much greater when cats are able to come into contact with other cats outside. Perhaps a lot of cats that did come outside in this study only stayed in a small range or even enclosed area around the house.

Visitation at pet shelters is not been investigated as a risk factor before, probably because population samples also contained cats from catteries [45, 46] and exact data on the difference between cats from domestic households and cats housed in large groups are not published.

Publications [35, 45, 46] do agree on the association between present signs of AORD and the presence of FCV of which two only found association with upper respiratory tract disease and one with oral lesions. This study investigated if there was a history of AORD and in contrast with another study [35] there was a positive association with FCV infection which is very likely since cats can stay long-term carriers of FCV after infection [25, 26].

Reports on age predisposition of a FCV infection are inconsistent [25, 35], but in this study an age predisposition for FCV was found in the categories of 1-3 years (OR=14.4) and 3-6 years (OR=19.2) in comparison to cats older than 12 years.

In this study most cats were vaccinated against FCV and FHV, around 70 to 80 per cent. Since vaccination does not protect cats against FCV infection, but only against the clinical signs [20, 33, 34], it is explainable why vaccination did not decrease the chance of carrying FCV in this study and others [45, 46]. However one study did find a smaller chance of FCV infection in vaccinated cats [35].

**Conclusion**

Our results demonstrate that FCV does play a role in the aetiology of FCGS, based on the high prevalence of FCV among cats with FCGS. However since other investigators have not been able to induce FCGS after inoculation of FCV [29-32, 39] a sole causative role can not be assigned to FCV alone. Probably the host's immune system plays a key role, either by a hyperactivity or a lack in its defence mechanisms [6, 8], but details are not well-known. More investigations on the role of the immune system in FCGS are necessary. Perhaps investigations around the different pathways of the immune system in cats with FCGS could elucidate whether cats with FCGS have a hyperactive or a deficient immune response.

At first site the prevalence of FCV among healthy cats in the Netherlands seems to be low (4%), but a much greater and more disperse sample from the Dutch cat population should be examined to be more certain.

Results in this study suggest that in order to decrease the chance of cats developing acute and/or chronic oral/respiratory problems cat owners can be advised to vaccinate their cats against FCV properly, not to get many cats in their household and especially keep the cats out of a pet shelter as much as is possible.

Perhaps there should also be more research on the possibility of FCGS being hereditary in purebred cats, since the prevalence of FCGS in these cats is high.

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# Appendix I

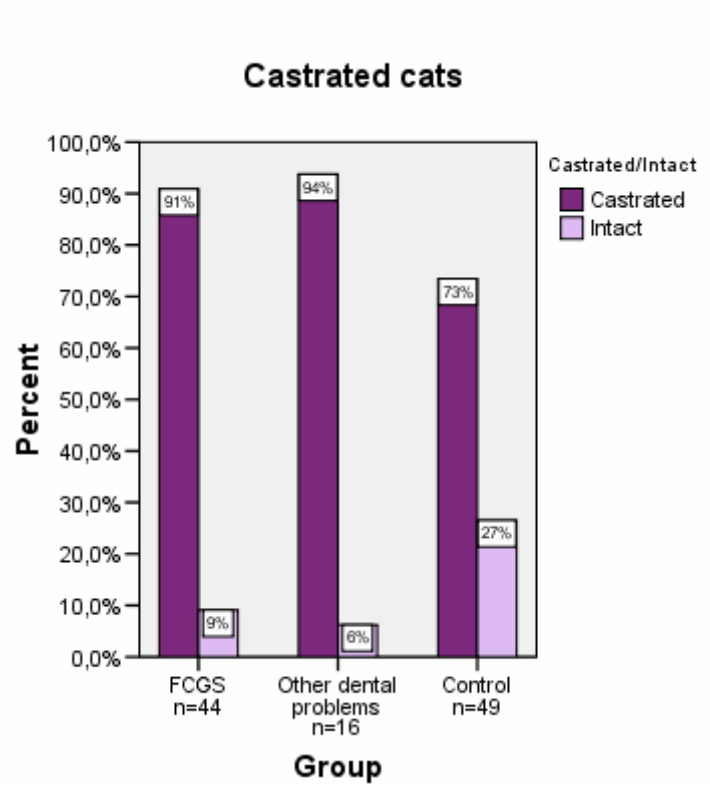


***Figure 11.*** *Percentages of cats castrated and intact.*

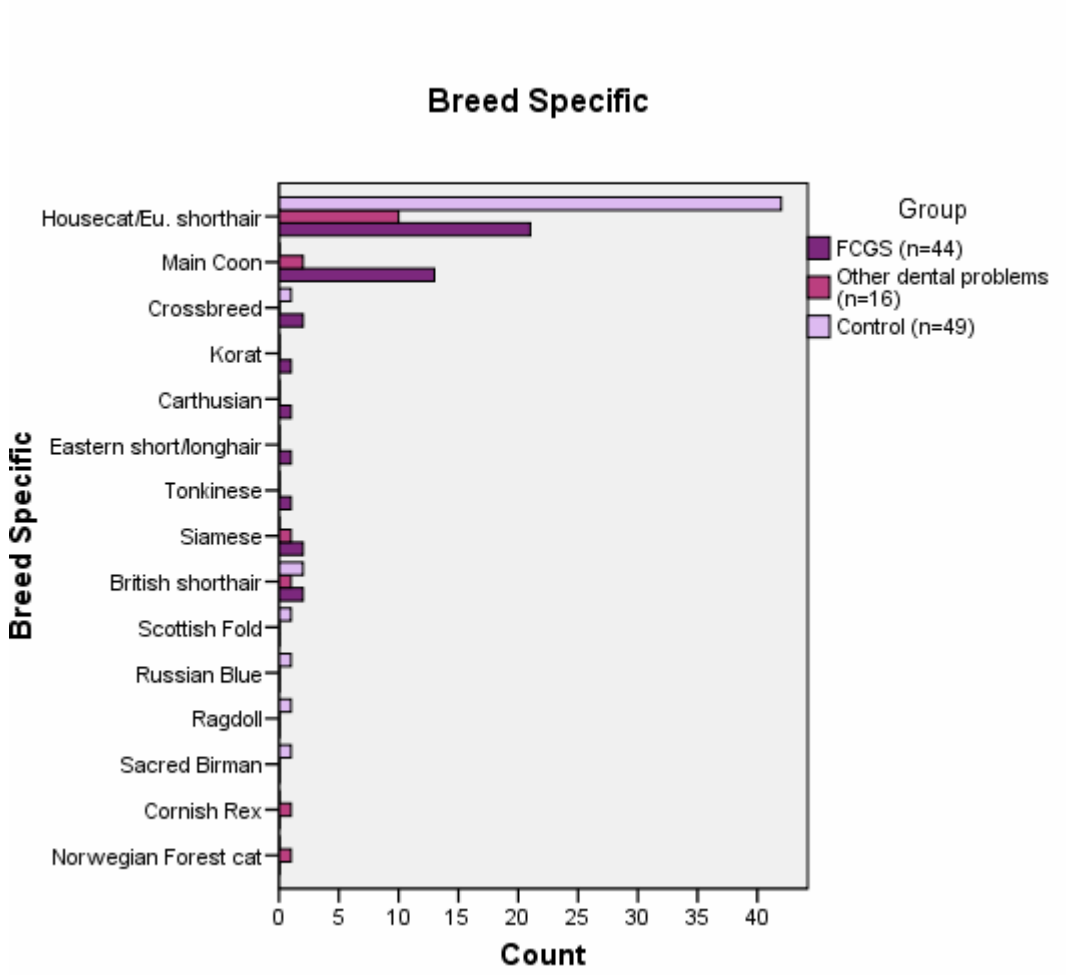


***Figure 12.*** *Specific breed representation.*

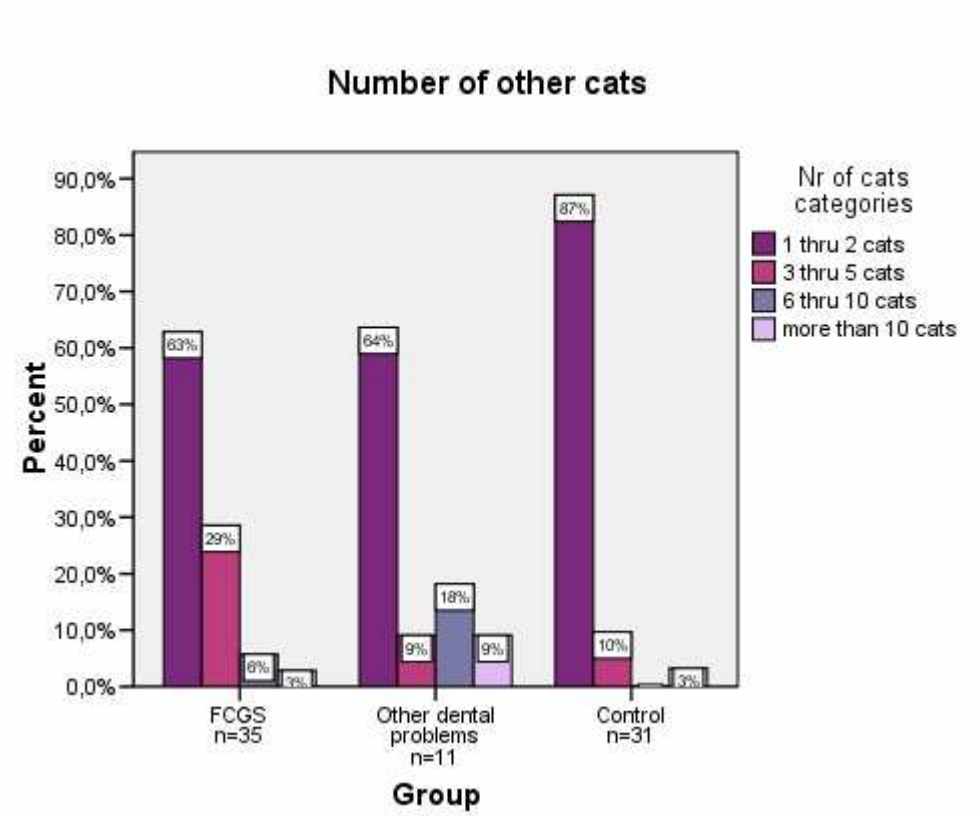


***Figure 13.*** *Number of other cats in the multicat households.*

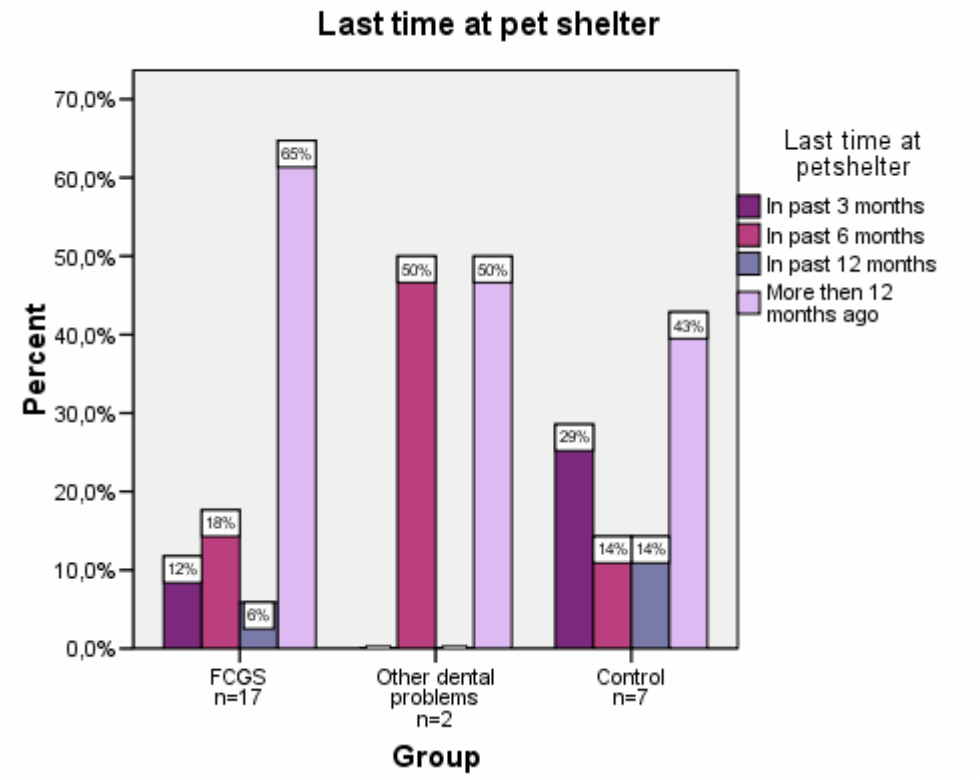


***Figure 15.*** *Last time the cats visited a pet shelter.*

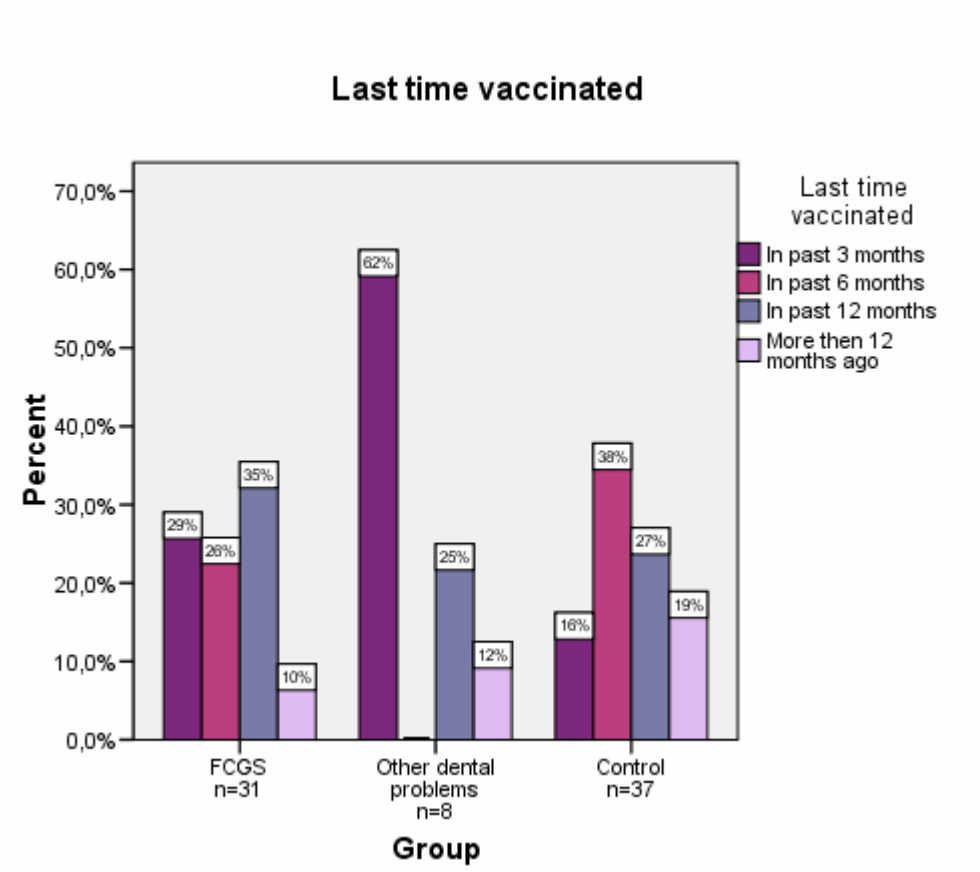


***Figure 14.*** *Last time vaccinated cats were vaccinated.*

# Appendix II

**Table 4.** Univariate associations between variables and the presence of FCGS, with a $Chi^2$ P value of ≤0.25.

| Variable | FCGS frequency | $Chi^2$ value | df | OR | CI 95% | P value |
|---|---|---|---|---|---|---|
| **FCV** | | 77.646 | 1 | | | <0.001# |
| Positive* | 42/44 (95.5%) | | | 493.500 | 66.544 – 3659.872 | <0.001 |
| Negative (R) | 2/49 (4.1%) | | | 1 | | |
| **Age** | | 18.470 | 5 | | | 0.002# |
| < 1 year | 3/23 (13.0%) | | | 0.188 | 0.031 – 1.122 | 0.067 |
| 1-3 years | 14/20 (70.0%) | | | 2.917 | 0.574 – 14.824 | 0.197 |
| 3-6 years | 11/16 (68.8%) | | | 2.750 | 0.509 – 14.860 | 0.240 |
| 6-9 years | 3/8 (37.5%) | | | 0.750 | 0.107 – 5.238 | 0.772 |
| 9-12 years | 9/17 (52.9%) | | | 1.406 | 0.277 – 7.131 | 0.681 |
| >12 years (R) | 4/9 (44.4%) | | | 1 | | |
| **Sex** | | 11.052 | 1 | | | 0.001# |
| Male* | 33/53 (62.3%) | | | 4.350 | 1.788 – 10.581 | 0.001 |
| Female (R) | 11/40 (27.5%) | | | 1 | | |
| **Breed** | | 15.442 | 2 | | | <0.001# |
| Purebred* | 21/27 (77.8%) | | | 7.000 | 2.455 – 19.957 | <0.001 |
| Crossbred | 2/3 (66.7%) | | | 4.000 | 0.343 – 46.676 | 0.269 |
| Domestic (R) | 21/63 (33.3%) | | | 1 | | |
| **Castrated/Intact** | | 4.720 | 1 | | | 0.030# |
| Yes* | 40/76 (52.6%) | | | 3.611 | 1.079 – 12.082 | 0.037 |
| No (R) | 4/17 (23.5%) | | | 1 | | |
| **Other cats in household** | | 13.051 | 2 | | | 0.001# |
| Yes* | 36/67 (53.7%) | | | 5.226 | 1.598 – 17.093 | 0.006 |
| No (R) | 4/22 (18.2%) | | | 1 | | |
| Unknown | 4/4 (100.0%) | | | | | |
| **Number of other cats** | | 6.059 | 3 | | | 0.109# |
| 1-2 cats | 22/49 (44.9%) | | | 0.815 | 0.048 – 13.785 | 0.887 |
| 3-5 cats | 10/13 (76.9%) | | | 3.333 | 0.157 – 70.906 | 0.440 |
| 6-10 cats | 2/2 (100.0%) | | | † | | |
| >10 cats (R) | 1/2 (50.0%) | | | 1 | | |
| **Outside** | | 6.052 | 2 | | | 0.049# |
| Yes | 23/54 (42.6%) | | | 0.835 | 0.352 – 1.978 | 0.681 |
| No (R) | 16/34 (47.1%) | | | 1 | | |
| Unknown | 5/5 (100.0%) | | | | | |
| **Pet shelter** | | 15.417 | 2 | | | <0.001# |
| Yes* | 18/25 (72.0%) | | | 5.271 | 1.894 – 14.673 | 0.001 |
| No (R) | 20/61 (32.8%) | | | 1 | | |
| Unknown | 6/7 (85.7%) | | | | | |
| **AORD** | | 30.153 | 2 | | | <0.001# |
| Yes | 15/15 (100.0%) | | | † | | |
| No (R) | 20/67 (29.9%) | | | 1 | | |
| Unknown | 9/11 (81.8%) | | | | | |

# P value according to Pearsons $Chi^2$ test

† Percentages not compatible for OR calculation

* Association statistically significant (P≤0.05)

(R) Reference category

**Table 5.** Multivariate associations between variables and the presence of FCGS using binary logistic regression analysis

| Variables | Regression coëfficiënt | S.E. | OR | CI 95% | P value |
|---|---|---|---|---|---|
| **Age** | | | | | |
| < 1 year* | -3.473 | 1.702 | 0.031 | 0.001 – 0.872 | 0.041 |
| 1-3 years | 0.094 | 1.370 | 1.098 | 0.075 – 16.119 | 0.945 |
| 3-6 years | 0.236 | 1.384 | 1.266 | 0.084 – 19.089 | 0.865 |
| 6-9 years | -1.069 | 1.604 | 0.343 | 0.015 – 7.957 | 0.505 |
| 9-12 years | -0.571 | 1.451 | 0.565 | 0.033 – 9.716 | 0.694 |
| >12 years (R) | | | 1 | | |
| **Sex** | | | | | |
| Male* | 1.419 | 0.726 | 4.134 | 0.997 – 17.135 | 0.050 |
| Female (R) | | | 1 | | |
| **Breed** | | | | | |
| Purebred* | 3.229 | 1.140 | 25.244 | 2.704 – 235.671 | 0.005 |
| Crossbred | 0.154 | 1.826 | 1.167 | 0.033 – 41.820 | 0.933 |
| Domestic | | | 1 | | |
| **Other cats in household** | | | | | |
| Yes | 1.671 | 0.892 | 5.316 | 0.925 – 30.546 | 0.061 |
| No (R) | | | 1 | | |
| **Pet shelter** | | | | | |
| Yes* | 2.244 | 0.851 | 9.433 | 1.781 – 49.970 | 0.008 |
| No (R) | | | 1 | | |
| Constant | -3.057 | 1.312 | 0.047 | | 0.020 |

* Association statistically significant (P≤0.05)
(R) Reference category

**Table 6.** Univariate associations between variables and the presence of other dental problems, with a $Chi^2$ P value of ≤0.25.

| Variable | Freq. of other dental problems | $Chi^2$ value | df | OR | CI 95% | P value |
|---|---|---|---|---|---|---|
| **FCV** | | 12.481 | 1 | | | <0.001# |
| Positive* | 6/8 (75.0%) | | | 14.100 | 2.475 – 80.315 | 0.003 |
| Negative (R) | 10/57 (4.1%) | | | 1 | | |
| **Age** | | 9.228 | 5 | | | 0.100# |
| < 1 year | 1/21 (4.8%) | | | 0.125 | 0.009 – 1.671 | 0.116 |
| 1-3 years | 1/7 (14.3%) | | | 0.417 | 0.029 – 6.064 | 0.522 |
| 3-6 years | 4/9 (44.4%) | | | 2.000 | 0.244 – 16.362 | 0.518 |
| 6-9 years | 4/9 (44.4%) | | | 2.000 | 0.244 – 16.362 | 0.518 |
| 9-12 years | 4/12 (33.3%) | | | 1.250 | 0.164 – 9.538 | 0.830 |
| >12 years (R) | 2/7 (28.6%) | | | 1 | | |
| **Breed** | | 5.306 | 2 | | | 0.070# |
| Purebred* | 6/12 (50.0%) | | | 4.200 | 1.116 – 15.804 | 0.034 |
| Crossbred | 0/1 (0.0%) | | | † | | |
| Domestic (R) | 10/52 (19.2%) | | | 1 | | |
| **Castrated/Intact** | | 2.936 | 1 | | | 0.087# |
| Yes | 15/51 (29.4%) | | | 5.417 | 0.649 – 45.184 | 0.119 |
| No (R) | 1/14 (7.1%) | | | 1 | | |
| **Other cats in household** | | 11.546 | 2 | | | 0.003# |
| Yes | 11/42 (53.7%) | | | 3.194 | 0.635 – 16.052 | 0.159 |
| No (R) | 2/20 (10.0%) | | | 1 | | |
| Unknown | 3/3 (100.0%) | | | | | |
| **Number of other cats** | | 6.778 | 3 | | | 0.079# |
| 1-2 cats | 7/34 (20.6%) | | | 0.259 | 0.014 – 4.683 | 0.361 |
| 3-5 cats | 1/4 (25.0%) | | | 0.333 | 0.009 – 11.939 | 0.547 |
| 6-10 cats | 2/2 (100.0%) | | | † | | |
| >10 cats (R) | 1/2 (50.0%) | | | 1 | | |
| **Outside** | | 17.711 | 2 | | | <0.001# |
| Yes | 9/40 (22.5%) | | | 2.613 | 0.508 – 13.451 | 0.251 |
| No (R) | 2/20 (10.0%) | | | 1 | | |
| Unknown | 5/5 (100.0%) | | | | | |
| **Vaccination** | | 11.064 | 2 | | | 0.004# |
| Yes | 11/50 (22.7%) | | | † | | |
| No (R) | 0/8 (0.0%) | | | 1 | | |
| Unknown | 5/7 (71.4%) | | | | | |
| **Last time vaccinated** | | 8.954 | 3 | | | 0.030# |
| In past 3 months | 5/11 (45.5%) | | | 5.833 | 0.525 – 64.823 | 0.151 |
| In past 6 months | 0/14 (0.0%) | | | † | | |
| In past 12 months | 2/12 (16.7%) | | | 1.400 | 0.105 – 18.615 | 0.799 |
| More then 12 months ago (R) | 1/8 (12.5%) | | | 1 | | |
| **Pet shelter** | | 12.355 | 2 | | | 0.002# |
| Yes | 2/9 (22.2%) | | | 1.302 | 0.231 – 7.336 | 0.765 |
| No (R) | 9/50 (18.0%) | | | 1 | | |
| Unknown | 5/6 (83.3%) | | | | | |
| **AORD** | | 20.075 | 2 | | | <0.001# |
| Yes | 2/2 (100.0%) | | | † | | |
| No (R) | 8/55 (14.5%) | | | 1 | | |
| Unknown | 6/8 (75.0%) | | | | | |

# P value according to Pearsons $Chi^2$ test (R) Reference category

† Percentages not compatible for OR calculation * Association statistically significant (P≤0.05)

**Table 7.** Multivariate associations between variables and the presence of Other dental problems using binary logistic regression analysis

| Variables | Regression coëfficiënt | S.E. | OR | CI 95% | P value |
|---|---|---|---|---|---|
| **Age** | | | | | |
| < 1 year | -3.190 | 2.127 | 0.041 | 0.01 – 2.659 | 0.134 |
| 1-3 years | -18.777 | 11903.327 | <0.001 | | 0.999 |
| 3-6 years | -0.198 | 1.608 | 0.820 | 0.035 – 19.165 | 0.902 |
| 6-9 years | 0.776 | 1.358 | 2.173 | 0.152 – 31.127 | 0.568 |
| 9-12 years | 0.644 | 1.291 | 1.904 | 0.151 – 23.925 | 0.618 |
| >12 years (R) | | | 1 | | |
| **Breed** | | | | | |
| Purebred* | 2.929 | 1.463 | 18.705 | 1.064 – 328.938 | 0.045 |
| Crossbred | | | † | | |
| Domestic (R) | | | 1 | | |
| **Outside** | | | | | |
| Yes | -0.87 | 1.056 | 0.830 | 0.105 – 6.580 | 0.860 |
| No (R) | | | 1 | | |
| Constant | -1.456 | 1.394 | 0.233 | | 0.296 |

* Association statistically significant (P≤0.05)
† Percentages not compatible for OR calculation
(R) Reference category

**Table 8.** Univariate associations between variables and the presence of FCV, with a $Chi^2$ P value of ≤0.25.

| Variable | FCV frequency | $Chi^2$ value | df | OR | CI 95% | P value |
|---|---|---|---|---|---|---|
| **Clinical signs** | | 78.482 | 2 | | | <0.001# |
| FCGS* | 42/44 (95.5%) | | | 493.5 | 66.544 – 3659.872 | <0.001 |
| Other dental problems* | 6/16 (37.5%) | | | 14.100 | 2.475 – 80.315 | 0.003 |
| None (control) (R) | 2/49 (4.1%) | | | 1 | | |
| **Age** | | 13.133 | 5 | | | 0.022# |
| < 1 year | 6/24 (25.0%) | | | 0.889 | 0.176 – 4.478 | 0.886 |
| 1-3 years* | 14/21 (66.7%) | | | 5.333 | 1.069 – 26.613 | 0.041 |
| 3-6 years | 13/20 (65.0%) | | | 4.952 | 0.986 – 24.875 | 0.052 |
| 6-9 years | 4/12 (33.3%) | | | 1.333 | 0.223 – 7.980 | 0.753 |
| 9-12 years | 10/21 (47.6%) | | | 2.424 | 0.500 – 11.761 | 0.272 |
| >12 years (R) | 3/11 (27.3%) | | | 1 | | |
| **Sex** | | 8.609 | 1 | | | 0.003# |
| Male* | 36/62 (58.1%) | | | 3.264 | 1.462 – 7.287 | 0.004 |
| Female (R) | 14/47 (29.8%) | | | 1 | | |
| **Breed** | | 15.073 | 2 | | | 0.001# |
| Purebred* | 24/33 (72.7%) | | | 5.444 | 2.195 – 13.505 | <0.001 |
| Crossbred | 2/3 (66.7%) | | | 4.083 | 0.352 - 47.301 | 0.260 |
| Domestic (R) | 24/73 (32.9%) | | | 1 | | |
| **Other cats in household** | | 8.821 | 2 | | | 0.012# |
| Yes* | 40/78 (51.3%) | | | 4.000 | 1.358 – 11.785 | 0.012 |
| No (R) | 5/24 (20.8%) | | | 1 | | |
| Unknown | 5/7 (71.4%) | | | | | |
| **Number of other cats** | | 5.035 | 3 | | | 0.169# |
| 1-2 cats | 24/56 (61.5%) | | | 0.375 | 0.032 – 4.381 | 0.434 |
| 3-5 cats | 10/14 (25.6%) | | | 1.250 | 0.087 – 17.975 | 0.870 |
| 6-10 cats | 3/4 (7.7%) | | | 1.500 | 0.055 – 40.633 | 0.810 |
| >10 cats (R) | 3/3 (5.1%) | | | 1 | | |
| **Outside** | | 4.571 | 2 | | | 0.102# |
| Yes | 24/63 (38.1%) | | | 0.551 | 0.240 – 1.261 | 0.158 |
| No (R) | 19/36 (52.8%) | | | 1 | | |
| Unknown | 7/10 (70.0%) | | | | | |
| **Pet shelter** | | 6.075 | 2 | | | 0.048# |
| Yes* | 17/27 (63.0%) | | | 2.877 | 1.147 – 7.214 | 0.024 |
| No (R) | 26/70 (37.1%) | | | 1 | | |
| Unknown | 7/12 (58.3%) | | | | | |
| **AORD** | | 22.898 | 2 | | | <0.001# |
| Yes* | 16/17 (94.1%) | | | 34.000 | 4.257 – 271.533 | 0.001 |
| No (R) | 24/75 (32.0%) | | | 1 | | |
| Unknown | 10/17 (58.8%) | | | | | |

# P value according to Pearsons $Chi^2$ test

* Association statistically significant (P≤0.05)

(R) Reference category

**Table 9.** Multivariate associations between variables and the presence of FCV using binary logistic regression analysis

| Variables | Regression coëfficiënt | S.E. | OR | CI 95% | P value |
|---|---|---|---|---|---|
| **Age** | | | | | |
| < 1 year | 2.035 | 1.321 | 7.652 | 0.575 - 101.857 | 0.123 |
| 1-3 years* | 2.671 | 1.299 | 14.448 | 1.133 - 184.309 | 0.040 |
| 3-6 years* | 2.957 | 1.317 | 19.246 | 1.457 - 254.250 | 0.025 |
| 6-9 years | -0.237 | 1.365 | 0.789 | 0.054 - 11.452 | 0.862 |
| 9-12 years | 2.119 | 1.287 | 8.322 | 0.668 - 103.702 | 0.100 |
| >12 years (R) | | | 1 | | |
| **Sex** | | | | | |
| Male | 0.898 | 0.543 | 2.454 | 0.846 - 7.118 | 0.099 |
| Female (R) | | | 1 | | |
| **Other cats in houshold** | | | | | |
| Yes* | 1.931 | 0.802 | 6.895 | 1.431 - 33.230 | 0.016 |
| No (R) | | | 1 | | |
| **Pet shelter** | | | | | |
| Yes* | 1.437 | 0.697 | 4.207 | 1.073 - 16.490 | 0.039 |
| No (R) | | | 1 | | |
| **AORD** | | | | | |
| Yes* | 4.837 | 1.461 | 126.112 | 7.198 - 2209.527 | 0.001 |
| No (R) | | | 1 | | |
| Constant | -5.302 | 1.572 | 0.005 | | 0.001 |

* Association statistically significant ($P \leq 0.05$)
(R) Reference category