SHORT COMMUNICATION

Prevalence of feline calicivirus in cats with odontoclastic resorptive lesions and chronic gingivostomatitis

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Abstract

Feline odontoclastic resorptive lesion (FORL) and feline chronic gingivostomatitis (FCGS) are two of the most common diseases of the feline oral cavity. While evidence is emerging that FCGS is caused by gingival inflammation initiated and perpetuated by the oral microbiota, little is known in this regard for FORL. Feline calicivirus (FCV) has been associated with the presence of FCGS and is thought to play a role in the initiation of this disease. In this study, the incidence of FCV was investigated in cats with FORL and FCGS, and compared to unaffected controls. FCV was detected by viral culture. The incidence of FCV was as follows: 6 (24.0%) of 24 control cats, 9 (22.5%) of 40 cats with FORL and 15 (60.0%) of 25 cats with FCGS were positive for FCV. There was a significant difference in FCV incidence between all the groups (p=0.003) but none between the control group and the FORL group. However, significant differences were observed in the incidence of FCV between control and FCGS (p=0.010) and between FORL and FCGS (p=0.006). It is concluded that although FCV may be associated with FCGS, it appears unlikely to play a role in FORL.

Keywords: feline; resorptive lesion; gingivostomatitis; feline calicivirus; inflammation

Feline odontoclastic resorptive lesion (FORL) and feline chronic gingivostomatitis (FCGS) are two of the most common oro-dental diseases of cats. FORL affects more than 60% of cats over the age of 6 years of age and its incidence increases with age (Lyon, 1992; Reiter and Mendoza, 2002; Mestrinho et al., 2013). It is a progressive disease characterised by tooth resorption due to the destructive activity of odontoclasts. FORL manifests as erosion of the surface of the tooth at the gingival border, with loss of cementum and dentin that leads to eventual penetration of the pulp cavity. Enamel resorption can also occur, leading to tooth fracture. Resorbed cementum and dentin is replaced with bone-like tissue. FORL has been clinically and radiographically classified into five stages (Reiter and Mendoza, 2002), varying from stage 1 (mild dental hard tissue loss, with lesions extending into the cementum only) to stage 5 (no crown with only root remnants remaining). FORL causes pain, gingival inflammation, destruction of periodontal attachment and tooth loss. Since FORL is such a progressive disease, the only treatment currently available is tooth extraction.

FCGS causes a severe, painful inflammation of the oral cavity that can affect a variety of sites (White et al., 1992). In its most severe presentation, a proliferative and ulcerative inflammation is seen at the tissue lateral to the palatoglossal folds (fauces) and the mucosa overlying the premolar/molar area extending to the buccal mucosa (Hennet et al., 2011). A wide range of clinical symptoms are often observed, including weight loss, dysphagia, loss of grooming behaviour, excess salivation and halitosis (Bonello, 2007).

While recent work has focused on the involvement of the oral microbiota in FCGS (Dolieslager et al., 2011, 2013), comparatively little is known about the involvement of microbiota in FORL. We hypothesise that the oral microbiota may have an influence on the inflammatory immune response in FORL, possibly due to changes in the gingival micro-environment, as is the case for FCGS. In this study we compared the incidence of feline

calicivirus (FCV) infection in cats with FORL, FCGS and unaffected controls in order to determine whether FCV could be one of the initiating causes of FORL.

A total of 90 cats were recruited to the study from the Nestlé Purina PetCare facility (St. Joseph, MO, USA): 40 cats with FORL, 25 cats with FCGS and 25 unaffected control cats with no signs of oral disease. All cats were either neutered or spayed. Cats were housed indoors with natural lighting and exposure to natural light cycles in environmentally controlled rooms in groups based on gender and compatibility. Cats were provided environmental enrichment consisting of multiple perches, access to toys, and direct interaction with caretakers on a daily basis. Cats had *ad libitum* access to water and were fed to maintain an ideal body condition. The study was conducted in strict accordance with the guideline established by the Nestlé Purina PetCare (NPPC) Advisory Committee. Apart from the oral conditions described above, all cats were otherwise in good health and appearance. All cats had regular dental evaluations and professional cleanings. Presence or absence of FORL was determined by the presence of obvious erosion of enamel and dentine at the base of the tooth with localised gingival inflammation; FCGS was diagnosed when severe gingival inflammation of the gum and mucosa of the palatoglossal folds were observed without signs of FORL. The presence of FCV virus in oral samples of all cats was determined by culture. In brief, supragingival plaque samples were collected with a swab, and dispersed in viral transport medium. Following collection, 200 μl of the transport medium was applied to a confluent monolayer of Crandall Rees feline kidney cells and incubated for 1 hour at 37ºC. The transport medium was removed, replaced with growth medium and cells incubated at 37ºC for 24 hours (Bidawid et al., 2003). Monolayers were examined daily for six days using an Olympus CK2 inverted microscope at 40x magnification and those showing cytopathic effects were stained with a fluorescein-labelled mouse anti-FCV antibody (United States Biologicals, Salem, USA) and visualised using a Leitz Diaplan fluorescent microscope

at 250x magnification; stained samples were deemed positive for FCV. All analysed samples were accompanied by both positive and negative controls. Statistical analysis was performed with GraphPad Prism 5.02 for Windows (GraphPad Software, San Diego CA, USA, www.graphpad.com) and consisted of a Fisher's exact test on a contingency table and ANOVA. Statistical significance was set at $p<0.05$.

Signalment of the cats used in the study is shown in Table 1. Due to the mixture of breeds within the FCGS group, the Fisher's exact test indicated a significant difference in the breeds of cats included in the study groups ($p=0.010$). However, this was unlikely to have impacted on the study since the proportion of FCV positive Scottish fold cats was two out of four tested, compared with 13 out of 21 shorthair cats tested.

All cats were greater than 9 months of age with a mean age of 6.1 years. The three cohorts comprised a total of 54 male and 36 female cats. The control group comprised 14 males and 11 females and the average age of these cats was 4.9 years (range 1.7 to 7.5 years); the FORL group comprised 28 males and 12 females and the average age of these cats was 7.2 years (range 2.9 to 11.4 years); the FCGS group comprised 12 males and 13 females and the average age of these cats was 6.4 years (range 0.9 to 14.9 years). ANOVA indicated that the unaffected control cats were significantly younger than the cats with FORL ($p=0.005$) but not the cats with FCGS. There was also no significant difference in the mean age of FORL and FCGS groups. If FCV carriage was directly influenced by age a greater carriage rate would be expected in the FORL group, but this was not the case. Despite the apparent discrepancy in gender distribution, there were no significant differences between the control, FORL and FCGS groups.

Of the 25 unaffected control cats, 6 (24%) cats were positive for FCV. Of the 40 FORL cats only 9 (22.5%) were positive for FCV while in the FCGS group 15 (60%) cats were positive for FCV. Fisher's exact test indicated a significant difference in FCV incidence

between all the groups (p=0.003). There was no significant difference between the control group and the FORL group with regard to the incidence of FCV. However, when the control group was compared with the FCGS group, a significant difference in the incidence of FCV was observed (p=0.010) and when FORL was compared with FCGS a significant difference was also observed (p=0.006). However, in case a potential gender imbalance could have influenced the result, the analysis was repeated on the male cats and female cats separately. Fisher's exact test indicated statistically significant differences in their FCV status between the three groups ($p<0.05$) and principally between the FORL and FCGS groups ($p<0.05$).

Our data confirms earlier studies which show a strong association of FCV with FCGS. Addie et al. (2003) showed that FCV shedding in a cat with FCGS ceased following an 11-month treatment regime to treat FCGS with thalidomide and lactoferrin. FCV RNA was detected in 17 (40.5%) of 42 cats with FCGS but in none of 19 healthy controls (Dowers et al., 2010). Virus testing, using the approach in the current study, identified FCV in 22 (71%) of 31 cats with FCGS but in only 2 (13.3%) of 15 healthy controls (Dolieslager, 2012). In the current study, somewhat surprisingly, FORL cats had a lower incidence of FCV (22.5%) than the unaffected control group (24%). Only one previous study has investigated a link between viral infection and FORL (Hofmann-Lehmann et al., 1998); it was observed that FORL lesions were more frequent in cats positive for feline immunodeficiency virus than normal controls. The causes of FORL appear to involve gingival inflammation and are likely to be multifactorial, as is the case for FCGS, and bacterial and/or viral infections (such as FCV) may trigger this inflammatory process that eventually leads to FORL. In support of this hypothesis, investigation of cytokine expression in FORL demonstrated elevated levels of the cytokines IL-1ß and IL-6 in the ground teeth of cats with FORL compared to normal teeth, suggesting that they may play a role in mediation of osteoclast activity in FORL (De Laurier et al., 2002). The same study also suggested that osteoprotegerin may have an inhibitory

effect on tooth resorption. However, our current study suggests that FCV is unlikely to have a causative effect in FORL. Like for FCGS, the mechanisms leading to FORL are multifactorial and studies are currently ongoing to identify the bacteria associated with FORL and whether they are able to initiate the disease process via a gingival inflammatory response.

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Table 1 Signalment of cats used in the study

	Control (n=25)	FORL (n=40)	FCGS (n=25)
Sex (%)	14M, 11F (56%M, 44%F)	28M, 12F (70%M, 30%F)	12M, 13F (48%M, 56%F)
Mean age (range) in years	4.9 (1.7–7.5)*	7.2 (2.9–11.4)†	6.4 (0.9–14.9)
Breed	DSH (25)	DSH (40)	DSH (21)* SF (4)

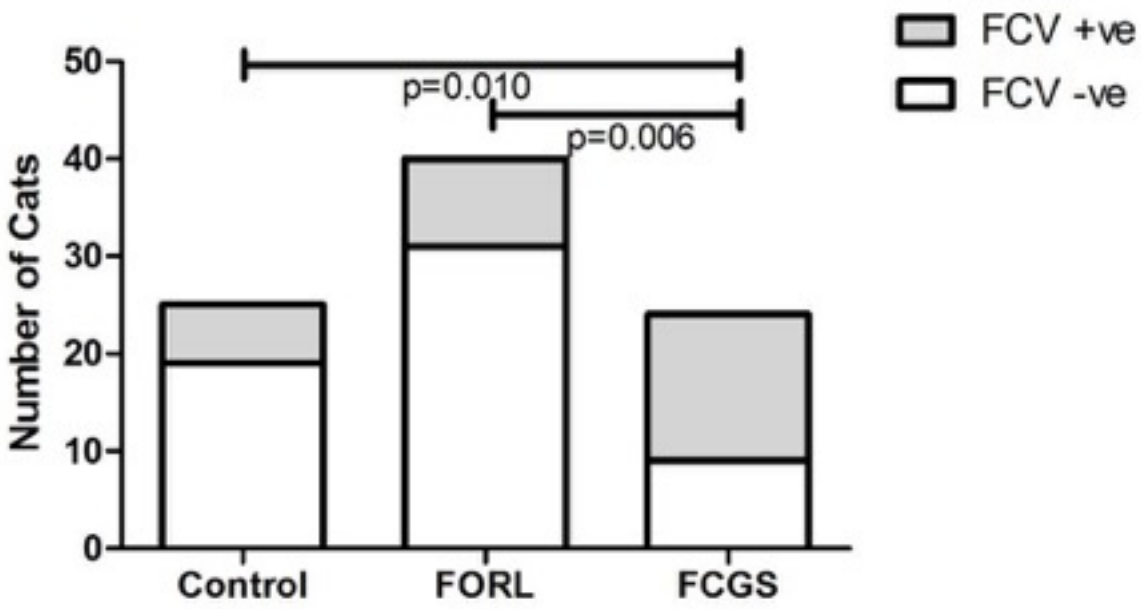
All animals were either neutered (male) or spayed (female) with the exception of one female cat in the FCGS group.

M, male; F, female; DSH, domestic shorthair; SF, Scottish fold.

*Significantly different from the FORL group.

† Significantly different from the control group.

Figure 1 Incidence of FCV in cats with FORL, FCGS and unaffected controls



The bars show the combined numbers of cats in each group. Shaded bars represent the number of cats within each group that were positive for FCV; the unshaded bars represent the number of cats within each group that were negative for FCV. Statistically significant differences in the presence of FCV in cats is indicated by the bars spanning the respective groups.