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1 **Identification of bacteria associated with feline chronic gingivostomatitis**
2 **using culture-dependent and culture-independent methods**
3
4

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18

19 **Abstract**

20 Feline chronic gingivostomatitis (FCGS) is a chronic inflammatory disease of the oral cavity
21 that causes severe pain and distress. There are currently no specific treatment methods available
22 and little is known regarding its aetiology, although bacteria are thought to play a major role. The
23 purpose of this study was to identify the oral bacterial flora in normal and diseased cats. Oral
24 swabs were obtained from the palatoglossal folds of eight cats (three normal, five FCGS) and
25 were subjected to microbiological culture. *Pasteurella pneumotropica* and *Pasteurella multocida*
26 subsp. *multocida* were the most prevalent species identified by culture methods in the normal
27 and FCGS samples, respectively. Bacteria were also identified using culture-independent
28 methods (bacterial 16S rRNA gene sequencing). For the normal samples, 158 clones were
29 analysed and 85 clones were sequenced. *Capnocytophaga canimorsus* (10.8% of clones
30 analysed) was the predominant species. Uncultured species accounted for 8.2% of clones
31 analysed, and 43.7% of clones analysed represented potentially novel species. For the FCGS
32 samples, 253 clones were analysed and 91 clones were sequenced. The predominant species was
33 *Pasteurella multocida* subsp. *multocida* (51.8% of clones analysed). Uncultured species
34 accounted for 8.7% of clones analysed and 4.7% of clones analysed represented potentially novel
35 species. It is concluded that oral flora in cats with FCGS appears to be less diverse than that
36 found in normal cats. However, *Pasteurella multocida* subsp. *multocida* is found to be
37 significantly more prevalent in FCGS than in normal cats and consequently may be of
38 aetiological significance in this disease.

39

40 Keywords: feline gingivostomatitis / bacteria / microbiological culture / 16S rRNA /
41 polymerase chain reaction

42 1. Introduction

43

44 Feline chronic gingivostomatitis (FCGS) is a severe inflammation of the feline oral cavity
45 that causes much pain and distress that can lead to euthanasia of affected animals (White et al.,
46 1992; Diehl and Rosychuk, 1993; Healey et al., 2007). The syndrome presents as a proliferative
47 and ulcerative inflammation of the oral cavity, mostly on the palatoglossal folds (often referred
48 to as the fauces) and the buccal gingiva. Other areas that can be affected are the pharynx, tongue
49 and lips. The palate can become inflamed at the sites of the molar and premolar teeth. Clinical
50 signs, generally caused by the inflammation which induces pain when opening the mouth, are
51 dysphagia, weight loss, loss of grooming behaviour, excessive salivation, pawing at the mouth
52 and halitosis (Bonello, 2007; Bellei et al., 2008).

53 FCGS is the most challenging of the oral inflammatory diseases to treat and its aetiology
54 remains unknown. Many different bacterial species, including *Prevotella* and *Porphyromonas*
55 species associated with human periodontal disease, have been implicated in FCGS (Mallonee et
56 al., 1988; Love et al., 1989). However, no reliable treatments or preventative measures are
57 available for the disease.

58 The purpose of this study was to identify the bacteria associated with FCGS, and with a
59 normal feline oral cavity, using both culture-dependent and culture-independent (bacterial 16S
60 rRNA gene sequencing) methods. The strength of culture-independent methods is that as well as
61 detecting cultivable bacteria they can also be used to identify bacteria that are uncultivable or
62 very fastidious in their growth requirements and, in addition, identify novel species.

63

64

65 2. Materials and methods

66

67 2.1. Sample collection and processing

68

69 Ethical approval was obtained from the Local Research Ethics Committee. Samples were
70 collected, using sterile swabs, from the palatoglossal folds of cats with a normal oral cavity
71 (three samples) which had been euthanatised for reasons unrelated to the oral cavity, and from
72 cats with FCGS (five samples). All cats were older than 18 months of age. Swabs were placed
73 into sterile reduced transport medium and immediately sent for laboratory analysis. Each swab
74 was immersed into 1.0 mL fastidious anaerobe broth (FAB) and mixed for 30 s to remove
75 bacteria.

76

77 2.2. Microbiological culture

78

79 Ten-fold serial dilutions (neat to 10^{-6}) of material eluted from each swab were prepared and
80 spiral plated onto both Columbia agar containing 7.5% (v/v) defibrinated horse blood (aerobic
81 culture) and FAA (BioConnections, Wetherby, UK) containing 7.5% (v/v) defibrinated horse
82 blood (anaerobic culture). Columbia blood agar plates were incubated in 5% CO₂ at 37°C, and
83 FAA plates were incubated at 37°C in an anaerobic chamber with an atmosphere of 85% N₂,
84 10% CO₂, and 5% H₂ at 37°C. Plates were incubated for up to seven days, and up to eight
85 morphologically distinct colonies were subcultured to obtain pure cultures. Isolates were
86 identified by 16S rRNA gene sequencing as described below.

87

88 2.3. DNA extraction

89

90 A crude bacterial DNA extract was prepared from each swab eluate by digestion with 1%
91 SDS and proteinase K (100 ug/mL) at 60°C for 60 min, followed by boiling for 10 min. DNA
92 was stored at -20°C until required. DNA was also extracted from bacterial isolates using the
93 same method.

94

95 2.4. PCR amplification of bacterial 16S rRNA genes

96

97 Bacterial 16S rRNA genes were amplified by PCR using universal primers. The primer
98 sequences were 5'-CAGGCCTAACACATGCAAGTC-3' (63f) and 5'-
99 GGGCGGWGTGTACAAGGC-3' (1387r) (Marchesi et al., 1998). Primers were synthesised
100 commercially (Sigma Genosys, Cambridge, UK). The PCR reactions were carried out in a total
101 volume of 50 µL containing 5 µL of the extracted DNA and 45 µL of reaction mixture
102 comprising 1 x GoTaq[®] PCR buffer (Promega, Southampton, UK) 1.25 U GoTaq[®] polymerase
103 (Promega), 1.5 mM MgCl₂, 0.2 mM dNTPs (New England Biolabs, Hitchin, UK), and each
104 primer at a concentration of 0.2 µM. The PCR cycling conditions consisted of an initial
105 denaturation phase of 5 min at 95°C, followed by 35 cycles of denaturation at 95°C for 1 min,
106 annealing at 58°C for 1 min and primer extension at 72°C for 1.5 min, and finally a primer
107 extension step at 72°C for 10 min.

108

109

110

111 2.5. *PCR quality control*

112

113 When performing PCR, stringent procedures were employed to prevent contamination.
114 Negative and positive controls were included with each batch of samples being analysed. The
115 positive control comprised a standard PCR reaction mixture containing 10 ng of *E. coli* genomic
116 DNA instead of sample; the negative control contained sterile water instead of sample. Each
117 PCR product (10 µL) was subjected to electrophoresis on a 2% agarose gel, and amplified DNA
118 was detected by staining with ethidium bromide (0.5 µg/mL) and examination under ultraviolet
119 illumination.

120

121 2.6. *Cloning of 16S rRNA PCR products*

122

123 PCR products were cloned into the cloning vector pSC-A-amp/kan using the StrataClone™
124 PCR Cloning Kit (Stratagene) in accordance with the manufacturer's instructions.

125

126 2.7. *PCR amplification of 16S rRNA gene inserts*

127

128 Following cloning of the 16S rRNA gene products amplified by PCR for each sample,
129 approximately 50 clones from each generated library were randomly selected. The 16S rRNA
130 gene insert from each clone was amplified by PCR with the primer pair 5'-
131 CCCTCGAGGTCGACGGTATC-3' (M13SIF) and 5'-CTCTAGA ACTAGTGGATCCC- 3'
132 (M13SIR). The M13SIF binding site is located 61 base pairs downstream of the M13 reverse

133 primer binding site, and the M13SIR binding site is located 57 base pairs upstream of the M13
134 –20 primer binding site, in the pSC-A-amp/kan cloning vector.

135

136 2.8. Restriction enzyme analysis

137

138 Each re-amplified 16S rRNA gene insert was subjected to restriction enzyme analysis.
139 Approximately 0.5 µg of each PCR product was digested in a total volume of 20 µL with 2.0 U
140 of each of the restriction enzymes *RsaI* and *MnII* (Fermentas Life Sciences, York, UK) at 37°C
141 for 1 h. Restriction fragments were visualised by agarose gel electrophoresis. For each library,
142 clones were initially sorted into groups based upon their *RsaI* restriction digestion profiles.
143 Further discrimination was achieved by digestion of clones with *MnII*, and clones with identical
144 restriction profiles for both enzymes were finally grouped together in distinct restriction
145 fragment length polymorphism (RFLP) groups.

146

147 2.9. DNA sequencing

148

149 The 16S rRNA gene insert of a single representative clone from each RFLP group was
150 sequenced. Sequencing reactions were performed using the SequiTherm EXCEL™ II DNA
151 Sequencing Kit (Cambio, Cambridge, UK) and IRD800-labelled 357f sequencing primer (5'-
152 CTCCTACGGGAGGCAGCAG-3') with the following cycling parameters: (i) initial
153 denaturation at 95°C for 30 s; (ii) 10 s at 95°C, 30 s at 57°C and 30 s at 70°C, for 20 cycles and
154 (iii) 10 s at 95°C and 30 s at 70°C for 15 cycles. Formamide loading dye (6 µL) was added to
155 each reaction mixture after thermal cycling and 1.5 µL of each reaction mixture was run on a LI-

156 COR Gene ReadIR 4200S automated DNA sequencing system (MWG Biotech, Milton Keynes,
157 UK).

158

159 *2.10. DNA sequence analysis*

160

161 Sequence data were compiled using LI-COR Base ImagIR 4.0 software, converted to FASTA
162 format and compared with bacterial 16S rRNA gene sequences from the EMBL and GenBank
163 sequence databases using the advanced gapped BLAST program, version 2.1 (Altschul et al.,
164 1997). The program was run through the National Centre for Biotechnology Information website
165 (<http://www.ncbi.nlm.nih.gov/BLAST>). Clone sequences that demonstrated at least 97% identity
166 with a known sequence from the database were considered to be the same species as the
167 matching sequence with the highest score. Sequences with less than 97% identity were classified
168 as potentially novel phylotypes.

169

170 **3. Results**

171

172 *3.1. Culture-dependent methods*

173

174 The bacterial isolates obtained by culture-dependent methods were identified by 16S rRNA
175 gene sequencing.

176 The bacteria identified from the three normal samples (N1 to N3) are shown in Table 1. A
177 total of 29 isolates were obtained and the most frequently isolated bacteria were *Pasteurella*
178 *pneumotropica* (3 isolates, 10.3%) and uncultured bacterium (3 isolates, 10.3%).

179 The bacteria identified from the five FCGS samples (F1 to F5) are shown in Table 1. A total
180 of 59 isolates were obtained and the most frequently isolated bacteria were *Pasteurella*
181 *multocida* subsp. *multocida* (11 isolates, 18.6%), uncultured bacterium (8 isolates, 13.6%) and
182 *Pasteurella multocida* subsp. *septica* (8 isolates, 13.6%). A further 6 isolates (10.2%) were
183 identified as either *Pasteurella multocida* subsp. *multocida* or *Pasteurella multocida* subsp.
184 *septica*.

185

186 3.2. Culture-independent methods

187

188 All three normal and five FCGS samples were positive for the presence of bacteria as
189 determined by 16S rRNA PCR analysis.

190 In total, 158 clones were analysed and 85 clones were sequenced across the three normal
191 samples. The bacteria identified (23 phylotypes) are grouped according to species in Table 2.
192 The predominant species was *Capnocytophaga canimorsus* (10.8% of clones analysed).
193 Uncultured species accounted for 13 (8.2%) of clones analysed.

194 In total, 253 clones were analysed and 91 clones were sequenced across the five FCGS
195 samples. The bacteria identified (19 phylotypes) are grouped according to species in Table 2.
196 The predominant species was *Pasteurella multocida* subsp. *multocida* (51.8% of clones
197 analysed). Uncultured species accounted for 22 (8.7%) of clones analysed.

198 In the normal samples, sixty-nine (43.7%) of clones analysed represented potentially novel
199 species (Table 3). In the FCGS samples, twelve (4.7%) of clones analysed represented
200 potentially novel species (Table 3).

201

202 4. Discussion

203

204 FCGS is a common and debilitating disease of unknown aetiology, although bacteria are
205 thought to play an important role in the disease process. Bacterial species which have been
206 implicated include *Bartonella* species and Gram-negative anaerobes. Initial small-scale
207 serological studies suggested a link between *Bartonella henselae* and FCGS (Ueno et al., 1996;
208 Glaus et al., 1997) but other larger-scale studies utilising a combination of ELISA, Western blot
209 immunoassay and PCR failed to find any correlation (Quimby et al., 2008; Dowers et al., 2009).
210 Serological responses to the Gram-negative anaerobes *Actinobacillus actinomycetemcomitans*,
211 *Bacteroides intermedius* and *Bacteroides gingivalis* have been demonstrated in cats with FCGS
212 (Sims et al., 1990) and several *Bacteroides* species have also been isolated from the oral cavity
213 (Love et al., 1989).

214 In the current study, we used molecular cloning and sequencing of bacterial 16S rRNA genes
215 (culture-independent methods), in tandem with conventional culture-dependent methods, to
216 identify the bacteria associated with FCGS and health. This is the first study to use such an
217 approach in an attempt to identify the microbial flora associated with FCGS and the healthy
218 feline oral cavity. The key finding of our study was that the proportion of *Pasteurella multocida*
219 subsp. *multocida* was greatly increased in FCGS compared with the healthy samples,
220 representing over half the identified microbial flora as determined by the culture-independent
221 approach. Therefore, this species may be considered to be of aetiological importance in FCGS.
222 *P. multocida* subsp. *multocida* is commonly found in the healthy feline oral cavity (Love et al.,
223 1990) and is associated with cat-bite infections (Love et al., 2000). The organism is also found in
224 feline periodontal disease, although its numbers decrease with increasing severity of the disease

225 (Mallonee et al., 1988). The massive overgrowth of *P. multocida* subsp. *multocida* in the FCGS
226 samples resulted in a dramatic reduction of some bacteria found at high levels in the normal
227 samples, most notably *C. canimorsus* and *Desulfomicrobium orale*, and this is most likely due to
228 increased competition for nutrients.

229 Overall, microbial diversity was less in the FCGS samples compared to the normal group.
230 Culture-independent methods identified 23 different phylotypes in the normal samples,
231 compared to 19 in the FCGS group. Uncultured bacteria were found at similar levels in the
232 normal and FCGS groups (8.2% and 8.7%, respectively). Potentially novel species were present
233 at significantly higher levels in the normal samples than in the FCGS group (43.7% and 4.7%,
234 respectively). The very high prevalence of potentially novel species in the normal samples is
235 perhaps unsurprising given that this is the first study to use culture-independent methods to
236 identify bacteria in the feline oral cavity. However, confirmation of such species as being novel
237 would require sequencing of the entire 16S rRNA gene.

238 The finding that *Pasteurella multocida* subsp. *multocida* was the predominant species
239 identified by culture-independent methods in the FCGS samples is corroborated by the culture
240 data obtained, with 18.6% of bacterial isolates from the FCGS group being identified as this
241 species. However, there were also some differences in the bacterial species identified by the
242 culture-dependent and culture-independent methods used in the current study. For example,
243 *Capnocytophaga canimorsus* was the predominant species identified by culture-independent
244 methods in the normal samples but was not isolated by the culture methods employed. One
245 possible reason for this is the use of standard culture media and incubation conditions, which
246 were used to ensure that as many types of bacteria as possible were cultured. However, this
247 approach may not have been suitable for the culture of fastidious species. This lends credence to

248 the suggestion that culture-independent methods should be conducted in parallel with the
249 conventional culture methods in order to identify as many bacterial species as possible in each
250 sample. Conversely, some bacteria were isolated by culture methods yet were not identified by
251 culture-independent methods. This could be attributed to the phenomenon of primer bias (Suzuki
252 and Giovannoni, 1996; Polz and Cavanaugh, 1998), which leads to unequal amplification of PCR
253 products and consequent inaccuracies in the true numbers of species present within the sample.

254 It is concluded that a wide range of bacteria are present in the healthy feline oral cavity.
255 However, the microbial diversity significantly decreases in cats with FCGS, in which the
256 predominant species is *Pasteurella multocida* subsp. *multocida*. This species is associated with
257 the normal feline oral flora but a huge increase in its prevalence in FCGS suggests that it may be
258 an important aetiological agent of this disease.

259

260 **Conflicts of interest**

261

262 The authors have no conflicts of interest.

263

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265

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Table 1 Bacterial species identified by 16S rRNA sequencing of isolates obtained following microbiological culture from three normal samples (N1 to N3) and five FCGS samples (F1 to F5).

Species	Normal	FCGS
	No. of isolates (% of total) <i>n</i> =29	No. of isolates (% of total) <i>n</i> =59
<i>Actinomyces canis</i>	2 (6.9)	
<i>Anaerococcus</i> sp. / <i>Peptostreptococcus</i> sp.*	1 (3.4)	
<i>Bacillus</i> sp.	1 (3.4)	
<i>Bacteroides tectus</i>		2 (3.4)
<i>Bergeyella</i> sp.	1 (3.4)	
<i>Catonella</i> sp.	1 (3.4)	
<i>Chryseobacterium</i> sp.		2 (3.4)
<i>Clostridium perfringens</i>	1 (3.4)	
<i>Corynebacterium felinum</i>	1 (3.4)	
<i>Cupriavidus basilensis</i>		1 (1.7)
<i>Cytophaga</i> sp.	1 (3.4)	
<i>Enterobacter</i> sp.	1 (3.4)	
<i>Enterococcus casseliflavus</i>	1 (3.4)	
<i>Enterococcus faecalis</i>		1 (1.7)
<i>Enterococcus</i> sp.	1 (3.4)	
Eubacteriaceae ¹ bacterium	1 (3.4)	
<i>Filifactor villosus</i>	2 (6.9)	
<i>Gemella palaticanis</i>		1 (1.7)
<i>Moraxella ovis</i>		1 (1.7)
<i>Mycoplasma arginini</i>	1 (3.4)	
<i>Neisseria</i> sp.	2 (6.9)	
<i>Pantoea agglomerans</i>	1 (3.4)	
<i>Pasteurella multocida</i> subsp. <i>multocida</i>		11 (18.6)
<i>Pasteurella multocida</i> subsp. <i>septica</i>	2 (6.9)	8 (13.6)
<i>Pasteurella pneumotropica</i>	3 (10.3)	5 (8.5)
<i>Pasteurella</i> sp.		1 (1.7)
<i>Pasteurella</i> subsp. <i>multocida</i> / <i>septica</i> *		6 (10.2)
<i>Porphyromonas</i> sp. (oral)		1 (1.7)
<i>Pseudomonas reactans</i>		1 (1.7)
<i>Pseudomonas</i> sp.		2 (3.4)
<i>Staphylococcus aureus</i>		1 (1.7)
<i>Staphylococcus</i> sp.		1 (1.7)
<i>Streptococcus minor</i>	1 (3.4)	
<i>Streptococcus sobrinus</i>		1 (1.7)
Uncultured bacterium	3 (10.3)	8 (13.6)
Uncultured <i>Haemophilus</i> sp.	1 (3.4)	
Uncultured <i>Micrococcus</i>		1 (1.7)
<i>Virgibacillus halophilus</i>		4 (6.8)

¹Family; *Unable to distinguish between two or more species, therefore grouped generically.

Table 2 Bacterial species (at least 97% identity) identified by 16S rRNA sequencing of clones from three normal control samples (N1 to N3) and five FCGS samples (F1 to F5).

Species	Normal		FCGS	
	No of clones analysed (% of total) <i>n</i> =158	No of clones sequenced (% of total) <i>n</i> =85	No of clones analysed (% of total) <i>n</i> =253	No of clones sequenced (% of total) <i>n</i> =91
<i>Abiotrophia defectiva</i>	1 (0.6)	1 (1.2)		
<i>Advenella</i> sp. C12 / <i>Pelistega europaea</i> / <i>Tetrathioabacter kashmirensis</i> *	1 (0.6)	1 (1.2)		
Bacterium cp04.13	3 (1.9)	3 (3.5)		
<i>Bacteroides tectus</i>			1 (0.4)	1 (1.1)
<i>Bergeyella</i> sp.	11 (7.0)	7 (8.2)		
<i>Capnocytophaga canimorsus</i>	17 (10.8)	2 (2.4)	1 (0.4)	1 (1.1)
<i>Capnocytophaga cynodegmi</i>			1 (0.4)	1 (1.1)
<i>Capnocytophaga</i> sp.	1 (0.6)	1 (1.2)		
<i>Citrobacter amalonaticus</i> / <i>Citrobacter</i> sp. R3*	1 (0.6)	1 (1.2)		
<i>Clostridium botulinum</i> / <i>Clostridium sporogenes</i> *	1 (0.6)	1 (1.2)		
<i>Comamonas</i> sp.	2 (1.3)	1 (1.2)		
<i>Desulfomicrobium orale</i>	9 (5.7)	2 (2.4)		
<i>Fusobacterium canifelinum</i>			4 (1.6)	4 (4.4)
<i>Lysobacter</i> sp.	1 (0.6)	1 (1.2)		
<i>Moraxella ovis</i>			3 (1.2)	2 (2.2)
<i>Pasteurella multocida</i> subsp. <i>multocida</i>	4 (2.5)	4 (4.7)	131 (51.8)	24 (26.4)
<i>Pasteurella multocida</i> subsp. <i>septica</i>	5 (3.2)	1 (1.2)	1 (0.4)	1 (1.1)
<i>Pasteurella pneumotropica</i>	4 (2.5)	4 (4.7)	11 (4.3)	4 (4.4)
<i>Pasteurella</i> sp.			1 (0.4)	1 (1.1)
<i>Pasteurella stomatis</i>	1 (0.6)	1 (1.2)		
<i>Pasteurella trehalosi</i>	1 (0.6)	1 (1.2)		
Pasteurellaceae ¹ bacterium R46	1 (0.6)	1 (1.2)		
<i>Peptococcus</i> sp. (oral)			24 (9.5)	7 (7.7)
<i>Peptostreptococcus</i> sp.	1 (0.6)	1 (1.2)		
<i>Porphyromonas cangingivalis</i>			1 (0.4)	1 (1.1)
<i>Porphyromonas canoris</i>			1 (0.4)	1 (1.1)
<i>Porphyromonas circumdentaria</i>			3 (1.2)	2 (2.2)
<i>Pseudomonas reactans</i>			13 (5.1)	6 (6.6)
<i>Pseudomonas</i> sp.			22 (8.7)	13 (14.3)
<i>Pseudomonas synxantha</i>			1 (0.4)	1 (1.1)
<i>Simonsiella</i> sp.	2 (1.3)	2 (2.4)		
Uncultured bacterium	12 (7.6)	7 (8.2)	14 (5.5)	8 (8.8)
Uncultured <i>Capnocytophaga</i> sp.			2 (0.8)	2 (2.2)
Uncultured Prevotellaceae ¹	1 (0.6)	1 (1.2)		
Uncultured <i>Pseudomonas</i> sp.			6 (2.4)	2 (2.2)
<i>Virgibacillus</i> sp. / <i>Salibacillus</i> sp.*	1 (0.6)	1 (1.2)		
Xanthomonadaceae ¹ bacterium	8 (5.1)	3 (3.5)		

¹Family; *Unable to distinguish between two or more species, therefore grouped generically.

Table 3 Potentially novel bacterial species (less than 97% identity) identified by 16S rRNA sequencing of clones from three normal samples (N1 to N3) and five FCGS samples (F1 to F5).

Most closely related species	Normal		FCGS	
	No of clones analysed (% of total) <i>n</i> =158	No of clones sequenced (% of total) <i>n</i> =85	No of clones analysed (% of total) <i>n</i> =253	No of clones sequenced (% of total) <i>n</i> =91
<i>Actinomyces</i> sp.	2 (1.3)	2 (2.4)		
<i>Bacteroides</i> sp. XB1A			2 (0.8)	1 (1.1)
<i>Capnocytophaga canimorsus</i>	9 (5.7)	3 (3.5)		
<i>Catonella</i> sp. (oral)			1 (0.4)	1 (1.1)
<i>Chryseobacterium</i> sp.	1 (0.6)	1 (1.2)		
<i>Eubacterium brachy</i>			2 (0.8)	1 (1.1)
<i>Mannheimia</i> sp.	1 (0.6)	1 (1.2)		
<i>Micromonas micros</i>			2 (0.8)	1 (1.1)
<i>Neisseria</i> sp. (oral)	1 (0.6)	1 (1.2)		
<i>Pasteurella multocida</i> subsp. <i>multocida</i>			1 (0.4)	1 (1.1)
<i>Pasteurella pneumotropica</i> / <i>Pasteurella stomatis</i> *			1 (0.4)	1 (1.1)
<i>Porphyromonas</i> sp. (oral)	10 (6.3)	5 (5.9)	1 (0.4)	1 (1.1)
<i>Prevotella</i> sp. (oral)	4 (2.5)	1 (1.2)		
Uncultured bacterium	16 (10.1)	10 (11.8)	2 (0.8)	2 (2.2)
Uncultured Bacteroidetes ² bacterium	3 (1.9)	1 (1.2)		
Uncultured <i>Capnocytophaga</i> sp.	10 (6.3)	6 (7.1)		
Uncultured <i>Catonella</i> sp.	2 (1.3)	1 (1.2)		
Uncultured Firmicutes ² bacterium	1 (0.6)	1 (1.2)		
Uncultured <i>Fusibacter</i> sp.	2 (1.3)	1 (1.2)		
Uncultured <i>Peptococcus</i> sp.	1 (0.6)	1 (1.2)		
<i>Virgibacillus marismortui</i>	6 (3.8)	2 (2.4)		

²Phylum; *Unable to distinguish between two or more species, therefore grouped generically.