1 **Quantitative sensory testing with Electronic von Frey Anaesthesiometer and von Frey**

2 **filaments in non-painful cats: a pilot study**

3 **Abstract**

4 **Objective** Measurement of sensory thresholds could represent a complementary tool to

5 behavioural pain scores in cats. The aim of this study was to investigate the feasibility of

6 quantitative sensory testing (QST) with the Electronic von Frey Anaesthesiometer (EVF) and

7 the von Frey filaments (VFF) in healthy cats, and to assess the limits of agreement (LOA)

8 between the two devices.

9 **Study design** Prospective clinical study.

10 **Animals** A total of 15 client-owned healthy cats.

11 **Methods** Two investigators (A and B) carried out the measurements independently. The EVF

12 and the VFF were applied on the upper lip and at the level of the medial aspect of the stifle. A

13 1-hour interval was allowed between the sets of measurements taken by investigators A and

14 B; each investigator repeated the entire session of measurements after 24 hours. The LOA

15 between the EVF and the VFF were analysed with the intra-class correlation coefficient

16 (ICC), and with the Bland Altman method.

17 **Results** Quantitative sensory testing with both the EVF and the VFF was feasible in healthy

18 cats; however, the willingness of the cats to cooperate was negatively affected by the

19 repetition of the measurements on the second day. The presence of the cat owners seemed to

20 facilitate the trial. There was a fair agreement between the EVF and the VFF (ICC = 0.49; CI:

21 0.13 – 0.70).

22 **Conclusions and clinical relevance** Our findings indicate that both EVF and VFF may be

23 used for QST in cats. Further trials will be needed to verify the usefulness of QST with EVF

24 and VFF in feline patients suffering from actual chronic pain.

25 *Keywords* Cat, Electronic von Frey, Pain, Quantitative sensory testing, von Frey Filaments

Introduction

Detecting and managing pain is an important duty for owners and veterinary professionals. Whilst acute postoperative pain can be easily anticipated and has, in most species, some recognizable features, chronic pain is subtle in nature and its recognition may be extraordinarily challenging, especially in cats. Chronic conditions that are common in cats (Klinck et al. 2012; Winer et al. 2016; and carry the potential for the development of chronic pain and central sensitization are feline chronic stomato-gingivitis (FCGS) and osteoarthritis (OA).

Quantitative sensory testing (QST) allows evaluation of the somatosensory function based on measurement of the mechanical sensory thresholds. Both the von Frey filaments (VFF) and the Electronic von Frey Anaesthesiometer (EVF) may be used for this purpose. The VFF are a set of 20 plastic monofilaments of progressively increasing thickness, which apply a force ranging from 0.008 to 300 grams on the body surface. The EVF represents the electronic version of the VFF and is composed of a control unit and a probe equipped with a rigid tip, capable of applying and measuring a force varying from 0 to 1000 grams. The force at which the target behavioural response is evoked – usually withdrawal or escape in non-verbal patients- is defined as threshold pressure. These devices are commonly used in human medicine to detect and quantify allodynia and hyperalgesia, as well as in laboratory rodents for sensory threshold testing (Lambert et al. 2009; Tena et al. 2010; Moore et al. 2013; Addison and Clements 2017). Recently, one study investigated the use of both VFF and EVF to quantify chronic pain associated to feline OA (Addison and Clements 2017).

The aims of the present study were:
- To investigate the feasibility of QST performed with the EVF and the VFF, applied at the stifle joint and at the upper lip of healthy cats, with the assumption that these

50   anatomical sites might be used in future trials to measure pain associated to OA and

51   FCSG, respectively; and

52   • To assess the limits of agreement (LOA) between EVF and VFF.

53   It was hypothesised that QST with both the EVF and the VFF is feasible in cats, and

54 that the LOA between the two devices would be high.

55

56 **Materials and Methods**

57 A total of 15 client-owned cats were enrolled in this trial. Exclusion criteria were the

58 presence of any disease that may cause pain, as well as any analgesic treatment that could

59 influence the response to the QST. This study was conducted under approval of the Clinical

60 Research Ethical Review Board of the Royal Veterinary College (license number: URN 2016

61 1647-3) and signed informed owner consent.

62   Two investigators (investigators A and B) carried out the measurements in a quiet

63 room of the hospital. Fifteen minutes of acclimatization, during which the cats were left

64 undisturbed to explore the environment, were allowed before commencing the measurements.

65 Cat owners were encouraged to attend the clinical trial, if they wished.

66   The cats were assessed with both the VFF (von Frey Filaments; Bioseb, France) and

67 the EVF (von Frey Anaesthesiometer Type 2390; IICT Life Science, CA, USA) while in a

68 standing or sitting position or sternal recumbency, either on a consult table or on the floor

69 depending on where physical restraint could be kept to a minimum. Both devices were

70 applied at two anatomical sites: the superior lip, at the level of the right canine tooth, and at

71 the medial aspect of the right stifle joint.

72   The investigators tested the two sites independently, always starting with the VFF.

73 The order by which the two sites were tested, as well as which investigator performed the

74 measurements first, was decided based on simple randomization (flipping of a coin). A time

75   interval of one hour was allowed between subsequent sets of measurements. Each

76   investigator tested both sites once a day. The entire sessions were then repeated after 24

77   hours, on day 2.

78   The measurements with the VFF were carried out as follows: filaments of

79   progressively increasing thickness, starting with 0.008 g, were applied consecutively to each

80   anatomical site, perpendicular to the skin surface, until either the filament bended or a

81   behavioral response was evoked. If the cat reacted to a specific filament with limb/head

82   withdrawal, head turning, watching the site of application, vocalization, hissing, or attempts

83   to bite/scratch, then the same filament was re-applied twice to verify that the behavioural

84   response was consistent. In order to avoid temporal summation, a minimal time interval of 30

85   seconds was allowed between subsequent applications (Nie et al. 2005). The size of the

86   filament that evoked a consistent behavioral response was recorded as threshold.

87   The measurements with the EVF were carried out as follows: the 1000 g probe was

88   equipped with the rigid tip. The latter was then perpendicularly applied to the skin surface of

89   the two sites of interest. The force of application was progressively increased until a

90   behavioral response could be evoked as for the VFF. As for the VFF, three subsequent

91   measurements were taken, with 30 second-interval between each. The mean of the obtained

92   values was recorded as threshold.

93

94   **Statistical analysis**

95   Normality of data was assessed with the D'Agostino, Skewness and Kurtosis tests.

96   The Spearman correlation coefficient (SCC) was used to analyse the degree of correlation

97   between the measurements obtained with the two devices. According to the guidelines

98   provided by the manufacturer of the statistic software used, for both analyses a result of 0 -0.19

99   was interpreted as very weak correlation, 0.20-0.39 as weak correlation, 0.40-0.59 as moderate

100 correlation, 0.60-0.79 as strong correlation; and 0.80-1.0 as very strong correlation (Systat

101 website, 2018). Additionally, the LA between the EVF and the VFF were analysed with the

102 intra-class correlation coefficient (ICC) and with the Bland-Altman analysis, used to define the

103 95% confidence intervals (CI; upper and lower bounds). The inter-device limits of agreement

104 were scored as follows: ICC < 0.40 = poor; ICC between 0.40 and 0.59 = fair; ICC between

105 0.60 and 0,74 = good; and ICC between 0.75 and 1= excellent (Cicchetti 1994). For the Bland-

106 Altman method, the number of standard deviations was set at 1.96, with 95% confidential

107 interval (Bland and Altman 1986).

108 Commercially available software were used (IBM SPSS Statistics 24, IBM

109 Corporation, NY, USA; and SigmaPlot 14 and SigmaStat 4, SYSTAT Software Inc, CA,

110 USA).

111

112 **Results**

113 A total of 15 mixed breed neutered cats, three females (20%) and 12 males (80%), aged 5.8 ±

114 4.7 years, were enrolled in the study. Only 4 out of the 15 cats were assessed in the presence

115 of their owner.

116 All the cats tolerated to complete one set of measurements. However, 11 cats became

117 less cooperative on day 2 and required a break longer than 30 seconds between subsequent

118 applications of the algometers. These cats were allowed to rest unrestrained for about 5

119 minutes. The remaining 4 cats were the ones whose owners were present throughout the

120 whole experimental session.

121 The data for VFF applied at the lip showed a two-sided distribution, with the 60 g and

122 300 g filaments being the sizes mostly recorded as threshold (10% and 44% of the cases,

123 corresponding to 6 and 25 out of 58 measurements, respectively). Regarding the

124   measurements carried out with the VFF at the stifle, the 300 g filament was recorded as

125   threshold in 87% of the cases (51 out of 58 measurements).

126   Overall, there was a fair agreement between the EVF and the VFF (ICC = 0.49; CI =

127   0.13 – 0.70; LOA = -188 +280; Bias = 47). The thresholds obtained for each pair of

128   measurements, as well as the values for SCC, ICC and 95% CI are presented in Table 1.

129

130   **Discussion**

131   The main finding of this study is that performing QST in cats with both the EVF and the VFF

132   is feasible, and that the sensory thresholds measured at the lip and at the stifle with these two

133   algometers are comparable, indicating a fair inter-device agreement. However, owing to

134   practicability, wider range of numerical outcome, and need for less subsequent applications,

135   the EVF may be regarded superior for QST in cats than its mechanical counterpart.

136   Our findings suggest that the uncooperative nature of cats may be exacerbated after

137   repeated assessments, a drawback that would affect feasibility after the first set of

138   measurements, and that time intervals longer than 24 hours may be needed between

139   subsequent evaluations. As the assessments progressed, most cats decreased their tolerance to

140   the procedure and returned inconsistent responses to the stimuli, sometimes anticipating the

141   application of the filament/probe with an escape reaction. On the other hand, some cats

142   showed instead some degree of habituation and seemed to get used to the measurements. In

143   these cats, the thresholds recorded on day 2 were higher than on day 1. These different

144   responses may be due to the personality of each individual cat.

145   Beside the repetition of the measurements and the individual personality of each cat,

146   other factors, such as the environment and the presence of the cat owner during the trial,

147   seemed to determine an effect on the attitude of the cats. The cats physically restrained by

148   their owner during the measurements were perceived as more cooperative and tolerant to the

149 procedure than those restrained by one of the investigators. Unfortunately, as most cat owners

150 were not willing to assist during the trial, this variable could not be standardized.

151 Nevertheless, it should be considered that the current study was designed with the purpose of

152 future applications for assessing chronic pain in clinical patients, which is more likely to

153 happen in a clinical scenario, without the cat owners being present.

154   The anatomical sites were chosen in perspective of possible future applications, being

155 the stifle and the lip commonly affected by conditions potentially associated to chronic pain

156 in cats. The choice of the lip as anatomical site for the measurements, however, posed some

157 important limitations. The presence of the whiskers, crucial for feline tactile perception

158 (Williams and Kramer 2010), may increase the sensitivity especially to the VFF, as the

159 smaller filaments may generate a prickling sensation, thus evoking a behavioural response

160 caused by discomfort rather than pain. Some of the cats enrolled in the study, indeed, showed

161 a rubbing gesture when the filaments were applied on the lips. Moreover, measuring

162 thresholds at the lip implies that the cat is able to see the probe, which may itself affect its

163 behavioral response. The medial aspect of the stifle posed some limitations as well, as this

164 area is difficult to reach in standing cats.

165   In most cats, at the level of the stifle the thickest VFF was recorded as threshold, and

166 this may be interpreted as a pitfall of the measuring instrument. There is, indeed, a large step

167 between the second to last and the last filaments (from 180 g to 300 g), which may jeopardize

168 the ability of the operator to detect small differences in thresholds. In that respect, EVF may

169 represent a better choice than the mechanical filaments in feline patients.

170   Another important limitation of the present study is the animal model used.

171 Presumably, the presence of chronic pain would decrease the sensory thresholds compared to

172 the study population, composed of healthy cats. This may allow the investigator to better

173 detect small differences in sensitivity between subjects, especially when using the VFF. Still

174   regarding the study population, a sample size calculation could have been performed by using

175   the means obtained from pilot measurements carried out by both operators before

176   commencing the trial. Since a pre-study trial could not be conducted, the number of cats to be

177   enrolled in the project was decided based on previously published literature. As a result, a too

178   small sample size cannot be excluded.

179

180   **Conclusion**

181   Quantitative sensory testing are feasible in cats with both the EVF and the VFF, as

182   long as the measurements are not repeated within a short time interval. Further trials are

183   needed to determine the usefulness of QST with EVF and VFF in feline patients suffering

184   from actual chronic pain.

185 **References**

186

187 Addison E, Clements D (2017) Repeatability of quantitative sensory testing in healthy cats in

188   a clinical setting with comparison to cats with osteoarthritis. J Feline Med Surg 19,

189   1274-1282.

190 Bland J, Altman D (1986) Statistical methods for assessing agreement between two methods

191   of clinical measurement. Lancet 1, 307–310.

192 Cicchetti DV (1994) Guidelines, criteria, and rules of thumb for evaluating normed and

193   standardized assessment instruments in psychology. Psychological Assessment 6, 284–

194   290.

195 Klinck M, Frank D, Guillot M et al. (2012) Owner-perceived signs and veterinary diagnosis

196   in 50 cases of feline osteoarthritis. Can Vet J 53, 1181–1186.

197 Lambert G, Mallos G, Zagami AS et al. (2009) Von Frey's hairs – a review of their

198   technology and use – a novel automated von Frey device for improved testing for

199   hyperalgesia. J Neurosci Methods 177, 420–426.

200 Moore S, Hettlich BF, Waln A (2013) The use of an electronic von Frey device for

201   evaluation of sensory threshold in neurologically normal dogs and those with acute

202   spinal cord injury. Vet J 197, 216–219.

203 Nie H, Arendt-Nielsen L, Andersen H (2005) Temporal summation of pain evoked by

204   mechanical stimulation in deep and superficial tissue. J Pain 2005; 6: 348-355.

205 Tena B, Escobar B, Arguis MJ et al. (2012) Reproducibility of electronic Von Frey and Von

206   Frey monofilaments testing. Clin J Pain 28, 318-223.

207 Williams C, Kramer EM, (2010) The Advantages of a Tapered Whisker. PLoS One 20, 5;

208   doi: 10.1371/journal.pone.0008806.PLoS One, 5, e8806.

209   Winer JN, Arzi B, Verstraete FJ et al (2016) Therapeutic Management of Feline Chronic

210   Gingivostomatitis: A Systematic Review of the Literature. Front Vet Sci 3, 54; doi:

211   10.3389/fvets.2016.00054http://doi.org/10.3389/fvets.2016.00054.

212   Website: Systat. https://systatsoftware.com/products/sigmastat/ Last accessed 7 February

213   2018.