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Approaching phantom complex after limb amputation in cats

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1 **Abstract**

2 The aims of this study was to describe the presence, clinical manifestations, risk factors,
3 quality of life and measurement of mechanical nociceptive threshold (MNT) of phantom limb
4 complex in a feline population that underwent amputation of a limb. A questionnaire was
5 developed containing 3 sections with a total of 71 closed-end questions. Clinical cases were
6 retrospectively reviewed. The evaluation of MNT was conducted applying an algometer at
7 the level of the stump of the amputated limb and exerting a gradually increasing pressure.
8 Descriptive statistics and frequency distribution analyses were performed on the collected
9 data. Chi-squared test or Fisher's exact test were used for assessment of the associations
10 between categorical variables. A total of 27 amputee cats were included in the study. All
11 owners answered the questionnaire, while the mechanical nociceptive threshold assessment
12 was conducted in 44% patients. The most frequent reason for amputation was related to
13 trauma. The presence of pain after limb amputation was commonly described by owners, and
14 the time between diagnosis and amputation was found to be significantly associated with the
15 presence of pain after amputation. The majority of owners described different manifestations
16 of pain or discomfort both before and after amputation, with environmental and physical
17 stress described as related to the onset of pain in some cases. Furthermore, a significant
18 reduction of the nociceptive threshold in the amputated region was highlighted.
19 This pilot study introduces previously unreported signs that may be interpreted as expressions
20 of pain in amputee cats.

21

22

23

24 *Keywords:* Cat; Pain; Phantom Limb Complex; Neuropathic pain; Quality of life

25

26 **Introduction**

27

28 Amputation of a limb is a procedure commonly performed on small animals (Kirpensteijn,
29 van den Bos, and Endenburg, 1999).

30 In humans, as a consequence of amputation, a syndrome called "phantom limb complex"
31 (PLC) can develop including signs of: (1) stump pain (SP), defined as pain localized in the
32 remaining post-amputation stump due to development of neuromas ; (2) phantom limb
33 sensation (PLS), defined as the perception of any sensation other than pain which originates
34 from the amputated limb; (3) phantom limb pain (PLP), defined as painful sensations
35 perceived in the area of the amputated limb (Hill, 1999; Nikolajsen and Jensen, 2001). These
36 three elements can coexist in the same patient and their differentiation in pets is particularly
37 challenging (Menchetti et al., 2017).

38 PLP occurs in 60-80% of patients in the first two years post-amputation and its onset usually
39 dates to the first post-amputation week (Nikolajsen and Jensen, 2000; Probstner et al., 2010).
40 Clinically, PLP may be confused with common postsurgical pain (PSP) as they might
41 temporally overlap. However, PSP progressively improves with wound healing, while PLP
42 worsens and evolves into chronic pain with lifelong persistence in 5-10% of cases (Melzack,
43 1971; Nikolajsen and Jensen, 2001).

44 In human medicine, the diagnosis of pain is based on verbal indicators and pain scales.
45 Furthermore, electrodiagnostic tests and quantitative sensory tests can be used to detect,
46 quantify and possibly monitor the presence of allodynia and hyperalgesia both in humans and
47 animals (Rolke, 2006; Dixon et al., 2007; Taylor et al, 2007; Harris et al., 2018; Hunt et al.,
48 2019).

49 In veterinary literature, studies have been carried out to investigate the degree of adaptation
50 to limb amputation, risk factors associated with poor quality of life and owner satisfaction

51 (Withrow and Hirsch, 1979; Kirpensteijn, van den Bos, and Endenburg, 1999; Dickerson et
52 al., 2015; Galindo-Zamora et al., 2016). A recent study described the occurrence of PLC
53 signs in dogs undergoing limb amputation. In particular, the duration of pre- amputation pain
54 and time between diagnosis and amputation were identified as risk factors for a higher
55 frequency of post-amputation pain episodes in amputated dogs (Menchetti et al., 2017). Post-
56 amputation limb behavior changes have been described in amputated cats (Forster et al.,
57 2010). However, aspects relating to the onset and semiology of these changes have not yet
58 been investigated. Despite a lack of evaluation scales for neuropathic pain in animals, direct
59 measurements for the investigation of thermal and mechanical nociceptive threshold have
60 been published (Dixon et al., 2007; Taylor et al, 2007; Harris et al., 2018; Hunt et al., 2019).
61 Whit specific reference to amputation, the measurement of mechanical nociceptive threshold
62 for the evaluation of post-amputation sensitization following tail docking in cow and swine
63 has been successfully applied (Di Gimignani et al., 2017; Troncoso et al., 2018).
64 The aims of the present study were to document the prevalence of PLC in a population of cats
65 with limb amputation, identifying signs and behaviors suggestive of neuropathic pain,
66 evaluate risk factors associated with PLC occurrence and determine the owners' perceptions
67 of the quality of life (QoL) of their 3-legged pets. Secondly, measurement of mechanical
68 nociceptive threshold was implemented in the clinical evaluation of selected patients for
69 objective identification of allodynia and/or hyperalgesia.

70

71 **Materials and Methods**

72

73 *Study population*

74 Clinical data of cats presented at the Veterinary Teaching Hospital (VTH) of the Department
75 of Veterinary Medical Sciences of the University of Bologna between January 2007 and
76 February 2018 were reviewed.

77 Inclusion criteria comprised cats that had undergone either complete or partial surgical
78 amputation of 1 limb at least 3-months before the survey, with complete medical records
79 including signalment (breed, sex, age, body weight) and detailed information about
80 amputation (cause, affected limb and level at which the amputation occurred, age of the
81 patient at the time of amputation and time elapsed between diagnosis and amputation). This
82 post-surgical interval of 3 months was considered an adequate period for a reliable
83 discrimination between PSP and allodynia and/or hyperalgesia associated with PLC.

84

85 *Questionnaire design and description*

86 A trial questionnaire was designed based on a study previously conducted on a canine
87 population that had undergone the amputation of a limb (Menchetti et al., 2017). WSAVA
88 guidelines for recognition, assessment and treatment of pain and studies on pain-related
89 behaviors in cats were consulted for identification of feline-specific behavioral indicators
90 related to the presence of pain (Waran et al., 2007; Holden et al., 2014; Mathews et al., 2014;
91 Merola & Mills, 2016).

92 The questionnaire included 3 sections with a total of 71 closed-end questions.

93 The first section consisted of 7 questions retrieving descriptive data about signalment, the
94 patient's environment, reason for amputation, and if the cat was owned at the time of the
95 amputation or was adopted soon after the injury.

96 The second section consisted of 59 questions intended to collect data regarding the pre- and
97 post -amputation periods, with special focus on pain characters, pain-related behaviors, post-
98 surgical complications, therapies and post-amputation Quality of Life (QoL). Pain was

99 characterized in terms of (1) *prevalence*, as pain observed by the owner before and after
100 amputation; (2) *onset*, as the time in which the cat started showing pain-related behaviors; (3)
101 *duration*, as time in which the pain-related behaviors persisted; (4) *frequency*, as pain
102 recorded episodes (several times daily, weekly, monthly); and (5) *type*, as pain quality
103 described as persistent, waxing and waning, or sudden and transient, referring to a 7-day pre-
104 amputation, a “typical week” and a “typical month” post-amputation (with “typical week”
105 and “typical month” referring to a representative time-frame of the ordinary pet’s life during
106 the pre- and post-amputation periods). Pain-related behaviors comprised both specific signs
107 of pain and more general behavioral changes (not necessarily related to pain) such as changes
108 in activity and social interactions, reduced food intake and sleep disturbances.

109 The last section consisted of 5 questions that evaluated the owner’s satisfaction regarding the
110 cat’s wellbeing and the impact of limb amputation on cat-owner relationship.

111 The questionnaire was administered to the owners by phone interview during February 2018.

112 Ethical approval was granted by the University of Bologna ethics committee (ID 664/2016).

113 An English translation of the original Italian version is available as supplementary material.

114

115 *Mechanical nociceptive threshold assessment*

116 The evaluation of mechanical nociceptive threshold (MNT) was conducted by a veterinary
117 specialist (GDR). After manual investigation of the stump, the MNT was measured with a
118 ProdPro® algometer (Topcat Metrology Ltd).

119 The measurement was carried out applying the algometer probe at the level of the stump of
120 the amputated limb and exerting a gradually increasing pressure. The algometer was removed
121 as soon as the patient showed signs of discomfort, such as withdrawal of the limb from
122 pressure or attempt to escape, and the maximum pressure applied was recorded. The same

123 procedure was also performed at the same level in the healthy contralateral limb. Three
124 measurements were made alternatively for each limb, and a mean value was then calculated.
125 Measurements were performed on cats that had a minimum post-surgical interval of least 3
126 months. Evaluation of the MNT was conducted upon owners' written informed consent as
127 required by the University of Bologna ethics committee (ID 664/2016).

128

129 *Statistical analysis*

130 Data analysis was performed using statistical analysis software (PAST 3.x The past of the
131 future, Hammer and Harper, Natural History Museum, University of Oslo, Oslo, Norway).
132 The contingency tables and graphs were obtained using an electronic spreadsheet (Microsoft
133 Excel, Microsoft Corporation, Microsoft Redmond campus, Redmond, Washington, United
134 States). The distribution characteristics of the values were checked for each linear parameter
135 by Shapiro-Wilk test and normal probability plotting. Contingency tables were generated for
136 the categorical variables (signalment, the pre- and post-amputation period questions and the
137 owner QoL satisfaction) and were described as percentages of the total respondents to each
138 individual question. The distribution of categorical variables was compared between dogs in
139 the pre- and post-amputation period by the chi-square test or Fisher's exact test depending on
140 whether the value in one or more of the cells of the contingency table was five or less. Data
141 regarding the nociceptive threshold measurements were compared by Student's t test.
142 p values were considered significant when < 0.05 .

143

144

145 **Results**

146

147 *Descriptive data*

148 A total of 27 cats that underwent limb amputation were included in the study. All owners
149 (27/27) answered the questionnaire, while the MNT assessment was conducted in 12/27
150 (44%) patients. At the time of the questionnaire, the majority of cats included in the study
151 were still alive (17/27; 63%).
152 All cats were European Shorthair (ESH) breed. Seventeen (63%) were males, 13 of which
153 (76%) neutered, whereas 10 (37%) were females, 5 of which (50%) spayed. At the time of
154 amputation, the median age was 5 years (range 4 months - 18 years) and the mean weight was
155 3.98 kg (\pm 0.35).

156 Twenty-two cats (81%) were already owned at the time of the amputation, while the
157 remaining 5 (19%) were traumatized cats and adopted shortly after the time of trauma.

158 At the time of investigation, most of the cats lived indoors (16/27; 59%), while the remaining
159 (11/27; 41%) had access to the garden.

160 The majority of cats (16/27; 59%) lived alone and unattended in the house from 4 to 8 hours a
161 day, some of them (8/27; 30%) were left companionless for less than 4 hours/day and the
162 remaining cats (3/27; 11%) were never alone in the house.

163 The main reason for amputation was trauma (17/27; 63%), followed by neoplasia (8/27;
164 29%), limb malformation (1/27; 4%) and infection (1/27; 4%).

165 Of the 27 cats, 12/27 (45%) underwent amputation of a thoracic limb, while 15/27 (55%)
166 underwent amputation of a pelvic limb. In 20/27 (74%) cats, the entire limb was amputated,
167 whereas the remaining 7/27 (26%) underwent partial amputation.

168

169 ***Owners' perception of pain prevalence, onset, duration, frequency and type***

170 According to the owners' perspective, pain was reported in 12/27 of cats before amputation
171 (44%) and in 11/27 of cats after amputation (41%)

172 Of cats showing pain before amputation, the majority (8/12; 67%) had a history of trauma.

173 The majority of owners noticed that cats experienced pain less than one month before
174 amputation (7/12; 58%).

175 After the amputation, the majority of owners (9/11; 82%) felt that their cats experienced pain
176 only in the post-surgical recovery period (from 24 hours to 1 week after amputation) and only
177 2/11 (18%) cats had pain protracted for more than one year after surgery.

178 According to our investigation, the time course of pain before surgery was not associated
179 with the development of postsurgical pain ($p= 0.09$; chi-square test, degrees freedom: 1).

180 Although not in the majority of cats, in a large percentage (10/27; 37%) the time elapsed
181 between the aetiological diagnosis and surgery ranged from one month to more than six
182 months. This factor was found to be significantly associated with the presence of pain after
183 amputation ($p= 0.04$; Fisher's exact test). Indeed, 7/11 (64%) of cats with post-surgical pain
184 showed the longest interval (from one to six months or more) between the time of diagnosis
185 and the amputation surgery.

186 Regarding the frequency of pre- and post-amputation pain episodes, no statistically
187 differences were observed ($p= 1$; Fisher's exact test), as cats experienced mostly daily
188 episodes of pain before (11/12; 92%) and after surgery (7/8; 87%).

189 Three of the 11 owners of cats experiencing pain after surgery were not able to answer the
190 question regarding the frequency of pain in the first week post-amputation, as those cats had
191 been hospitalized and this data was not available from medical charts.

192 Regarding the type of pain, during a "typical week" it was mostly described as "persistent"
193 (8/11; 73%). This data was not statistically different from a "typical month" after the
194 amputation (2/4; 50%) ($p= 0.06$; chi-square test, degrees freedom: 1).

195

196 ***Pain related manifestations and behavioral changes***

197 With respect to pain behaviors reported by the WSAVA guidelines, the majority of owners
 198 described several indicators of pain or discomfort both before (17/27; 63%) and after (23/27;
 199 85%) amputation (Supplementary Table 1). Pain behaviors showed by cats in the time frame
 200 comprised between 1 month and more than 1 year after amputation are showed in Table 1.
 201 This time frame was defined by the authors in order to avoid the post-surgical pain.
 202

Possible manifestations of pain	number/total (percentage)
Muscular twitching in the stump region	11/13 (85%)
Licking the stump	5/13 (38%)
Looking at the stump	5/13 (38%)
Restlessness	4/13 (31%)
Preferring to lie on a chilly floor	3/13 (23%)
Looking anxious	2/13 (15%)
Attitude of isolation	2/13 (15%)
Continuous change of position to find comfort	2/13 (15%)
Reluctance to move	1/13 (8%)
Biting and/or scratching the affected limb	1/13 (8%)
Vocalization	1/13 (8%)
Low ears	1/13 (8%)
Contracted cheeks	1/13 (8%)
Aggression toward animals	1/13 (8%)
Aggression toward humans	1/13 (8%)

203

204 Table 1: pain behaviors described by the owners in the timeframe comprised between 1

205

month and more than 1 year after amputation.

206

207 No statistically significant relation was found among the above pain behaviors after the
 208 amputation and their time frame of onset ($p= 0.5$; chi-square test, degrees freedom: 2) or
 209 duration ($p= 0.1$; Fisher's exact test). Despite it was not statistically significant ($p= 0.05$; chi-
 210 square test, degrees freedom: 1) a tendency toward a relation between the presence of pain
 211 before the amputation and the development of pain manifestations after amputation was
 212 observed.

213 Behavioral changes in terms of activity and withdrawal from interactions with humans and
 214 animals were described both before and after surgery (Table 2).

215

Changes in behavior	Pre- amputation number/total (percentage)	Post- amputation number/total (percentage)
Reduction of activity level	11/27; (41%)	3/27 (11%)
Appetite loss	5/27 (19%)	1/27 (4%)
Tendency to prevent contacts with humans and animals	3/27 (11%)	3/27 (11%)
Reduced sleep	3/27 (11%)	0/27 (0%)
Negative emotional state	/	3/27 (11%)
Reduced self-grooming	/	2/27 (7%)

216

217 Table 2: changes in behaviour before and after the amputation. /= the question was not
 218 included.

219

220 The presence of pain in the pre-amputation period was significantly related with the presence
 221 of behavioral changes ($p= 0.01$; chi-square test, degrees freedom: 1) and manifestations of
 222 pain or discomfort ($p= 0.01$; chi-square test, degrees freedom: 1) showed by cats before

223 surgery. Indeed, 8/11 (73%) cats with changes in behavior and 11/17 (65%) cats showing
224 signs of pain or discomfort in the pre-amputation period were also considered painful before
225 the amputation.

226 Accessory symptom that could possibly account for pain, were described by 9/27 owners
227 (33%) in the pre-amputation period and 5/27 owners (19%) in the post-amputation period.

228 Environmental factors and/or physical stress, as judged by the owner, were reported in
229 relation to the onset of pain in 4/27 cats (15%).

230

231 *Post-surgical complications*

232 Complications after surgery occurred in 3/27 (11%) cats, and comprised suture failure (3/3)
233 and wound infection (1/3). The presence of complications was not associated to the presence
234 of post-surgical pain ($p= 0.3$; chi-square test, degrees freedom: 1).

235

236 *Therapies*

237 Medical treatment before amputation was administered to 13/27 (48%) patients
238 (Supplementary Table 2).

239 In 8/13 (62%) cats, these treatments were administered for a period between 24 hours to 7
240 days. The administration of therapies before amputation was not statistically associated with
241 the occurrence of pain during the post-amputation period ($p= 0.07$; chi-square test, degrees
242 freedom: 1).

243 After amputation, treatments were administered to 23/27 cats (85%) (Supplementary Table
244 2).

245 When specifically asked about drugs administered because of pain after amputation, the most
246 frequent reported medications were anti-inflammatory drugs (14/27) followed by pain killers
247 (10/27), while specific treatments for neuropathic pain, such as gabapentin, were

248 administered in only one cat.

249

250 ***Owners' Quality of life perception***

251

252 The degree of adaptation after amputation was described from “good” to “excellent” in 26/27
253 (96%) cats, and 21/27 (78%) animals were able to ambulate within the first week after
254 amputation.

255 Without considering the first post-operative week, all owners described their cat’s quality of
256 life after amputation from “good” to “excellent”.

257

258 ***Owners' satisfaction and perspective***

259 After the amputation, the majority of owners (14/22; 64%) did not noted any modification in
260 the quality of their relationship with their pets despite the pre-amputation period (for this
261 question only owned cats before amputation were surveyed) and for 14/27 owners (52%) the
262 overall response of the family to the amputation was considered to be “very positive”.

263 However, during the first month after amputation, 5/27 (19%) owners felt their cat limited
264 their independence and 1/27 (4%) that his pet caused conflicts in his work or daily activities.

265 Nevertheless, the majority of owners (26/27; 96%) said they did not regret the decision to
266 have their pet amputated and all of them felt that they had been well informed by their
267 veterinarian during the decision-making process.

268

269 ***Mechanical nociceptive threshold assessment***

270 The stump palpation and the mechanical nociceptive threshold assessment made by the use of
271 a ProdPro® algometer was performed on 12/27 cats included in the study. Of these, one cat

272 was excluded from the measurements because of restlessness and aggressiveness, which
273 could have led to a biased assessment.

274 The measurements of the nociceptive threshold, performed in 11/27 cats (41%), revealed a
275 mean MNT of the amputated region of 6.3 ± 3.7 newtons, which was significantly lower than
276 that of the contralateral healthy limb (10.05 ± 3.5 newtons) ($p= 0.02$; Student's t test).

277

278

279 **Discussion**

280 The present investigation represents a preliminary step approaching PLC in cats after
281 amputation of a limb.

282 In human literature, the onset of chronic post-surgical pain is found in up to 60-80% of
283 patients during the first two years after the amputation of a limb (Nikolajsen and Jensen,
284 2000; Probstner et al., 2010). According to our survey, 41% of owners similarly felt that their
285 cats were in pain in the post-amputation period. In veterinary medicine, data from other
286 studies are in line with this result (Forster et al., 2010). According to Foster et al. (2010), 35%
287 of owners perceived their cat felt pain after discharge following limb amputation. In a
288 previous study conducted on a canine population after limb amputation, the presence of pain
289 in the post-amputation period was described by 85% of owners (Menchetti et al., 2017).

290 In human medicine, the onset of pain at the stump level is mostly found during the first week
291 after amputation and usually decreases with the healing of the surgical wound (Nikolajsen
292 and Jensen, 2001). However, in 5-10% of patients pain may persist over time and even
293 worsen, leading to neuropathic pain development (Nikolajsen and Jensen, 2001).

294 Despite the majority of owners in our study described the presence of pain during the first
295 week after surgery, a small number reported its persistence for more than one year. This
296 observation, while requiring a greater number of clinical cases to draw conclusions on, may

297 suggest that even in cats the onset of pain can occur months after surgery. In these subjects,
298 pain goes probably ahead its biological purpose (i.e. acute, inflammatory pain), and
299 neurological changes can occur leading to maladaptive (chronic, neuropathic) pain. As a
300 result, post-operative analgesic treatment might be extended for longer than strictly necessary
301 for wound healing, considering the long-term use of drugs aimed to prevent the occurrence of
302 neuropathic pain, and periodic assessment should cover a longer period than the usual 3-4
303 weeks. Similar findings were documented in a previously investigated canine population
304 (Menchetti et al., 2017) in which 79% of patients presented pain only in the first 4 weeks
305 following the surgery, while in 14% the pain occurred between one month and six months
306 after amputation.

307 Clinical assessment for allodynia and hyperalgesia at the stump level can be reliably
308 implemented in postoperative care via specific instrumental measurements (Fischer, 1998;
309 Hui et al., 2012). In the present study, the evaluation of the MNT showed a significant
310 reduction of the nociceptive threshold in the amputated region compared to the healthy
311 contralateral dermatomes. A similarly finding was obtained by Troncoso et al. (2018)
312 following the MNT evaluation at the stump level of docked tail with respect to intact tail in
313 cows. This result may be due to the establishment of synaptic changes and re-wiring of the
314 peripheral and central nervous system (neuroplasticity) following amputation (Flor, 2002;
315 Luo and Anderson, 2016; Collins et al., 2018). In veterinary medicine, there are currently no
316 studies that have objectively evaluated this event in companion animals that have undergone
317 limb amputation. Further studies are required for the integration of these measurements as
318 part of postoperative clinical care.

319 The role of the duration of pre-amputation pain in the development of PLC is still highly
320 debated. Specifically, a study conducted by Jensen et al. (1985) in human amputees
321 highlighted how the presence of pain for more than a month in the pre-amputation period can

322 be considered a risk factor for the development of PLC. However, this relationship was not
323 confirmed in other studies (Nikolajsen^a et al., 1997; Hanley et al., 2007).

324 In veterinary medicine, only one study investigated the effect of duration of pain before
325 surgery and PLC in a canine population, showing that the duration of pain before amputation
326 was related with high frequency (daily) of pain episodes after surgery, but was not related
327 with the presence of pain after surgery *per sé* (Menchetti et al., 2017). Results of the present
328 feline investigation are not in line with the canine data, as in cats there was not a relationship
329 between duration of pain before- and presence of pain after-amputation. In contrast with the
330 results obtained in the canine population (Menchetti et al., 2017), the surveyed feline
331 population did not show any difference in frequency of pain episodes, as they were mostly
332 daily both before and after the amputation.

333 In the present study, the time elapsed between diagnosis and amputation was associated with
334 the presence of pain in the post-amputation period. Specifically, the longer the time between
335 diagnosis and amputation, the greater the probability that the cat developed pain in the post-
336 amputation period. This correlation was not observed in a previous study on a canine
337 population (Menchetti et al., 2017).

338 Numerous studies have been conducted on human patients to evaluate the effects of
339 pharmacological treatments on the development of pain following surgery. Many of them
340 have shown that pain control in the pre-amputation period does not necessarily prevent the
341 development of pain in the post-amputation phase (Nikolajsen^b et al., 1997; Dahm et al.,
342 1998; Lambert et al., 2001). In line with this literature, in the present study there was no
343 significant relationship between the administration of pain control drugs in the pre-
344 amputation phase and the presence of pain in the post-amputation period. This data could
345 possibly suggest that there is a lack of correct management of the pain condition before the
346 surgery. However, this data remain to prove and there is need of more extensive large studies.

347 In our survey, owners reported behavioral modifications and changes in daily habits both
348 before and after amputation. Interestingly, similar changes have been previously described in
349 canine amputation patients, with modifications including aggression and anxiety
350 (Kirpensteijn et al., 1999; Menchetti et al., 2017). However, it is not possible to determine the
351 reason for these changes, as they could be related to the presence of pain, the change of
352 functional physical status due to a three legs condition or could not be related to the
353 amputation at all (Kirpensteijn et al., 1999; Menchetti et al., 2017).

354 In humans, the amputation of a limb has a negative impact on the everyday life of patients,
355 and the quality of life of amputated patients is lower when compared with the rest of the
356 general population (Pell et al., 1993; Sinha et al., 2011). In the present study, the quality of
357 life of amputee cats was perceived as good or excellent by the totality of owners. Similar data
358 was found in the study of a canine amputated population, in which 94% of owners defined
359 the quality of life of their pet as good and excellent following surgical intervention
360 (Menchetti et al., 2017). Differences emerged between human experience and the first
361 findings in veterinary medicine are perhaps due to the fact that animals have a less or no
362 negative perception of physical disability.

363 Following surgery, the majority of cats were given anti-inflammatories, analgesic or a
364 combination of them as analgesic therapy. However, 27% patients received no medications
365 for pain control. This data still highlights the lack of awareness of veterinarians and owners
366 regarding the need for pain relief after surgery. This may be due, in addition to the animal's
367 inability to verbalize, to the lack of knowledge and perception of pain related behaviors in
368 this species (Waran et al., 2007).

369 In veterinary medicine, the decision to have a pet amputated is often very difficult for the
370 owner, due to concerns that have mainly emotional and aesthetic implications (Withrow and
371 Hirsch, 1979; Kirpensteijn, van den Bos and Endenburg, 1999).

372 Previous studies investigated the satisfaction of the owners following the amputation,
373 highlighting that most of the owners did not regret their decision about amputation (Withrow
374 and Hirsch, 1979; Kirpensteijn, van den Bos and Endenburg, 1999; Forster et al., 2010;
375 Dickerson et al., 2015; Galindo-Zamora et al., 2016; Menchetti et al., 2017). In line with what
376 has been described before, the results of this study show that the majority of the cats' owners
377 did not regret this decision. These data further represent valuable references suggesting and
378 supporting the decision-making process towards amputation, often emotionally burdening for
379 the owners.

380 This study is the first attempt to identify and analyze the presence of pain and other clinical
381 signs related to PLC in amputated cats. Due to its nature, it presents with some limitations.
382 The restricted number of cases included prevents from further and wider generalization about
383 the clinical aspects described herein, but suggests at least a heightened attention for
384 behavioral changes in patients undergone amputation. Other limitations rely on the absence
385 of validated and objective scales for the assessment of pain in amputee dogs and cats.
386 Furthermore, the recognition of pain, especially in cats, is difficult due to the elusive nature
387 of its manifestation in the feline species and the lack of specific signs. Besides, the owner's
388 awareness of pain is subjective and may have partly influenced the results of the
389 questionnaire.

390 A larger study population, the development of validated scales and the serial execution of
391 instrumental measurements for the evaluation of the nociceptive threshold will allow in the
392 future to obtain more detailed information regarding the presence of pain in amputee cats.

393

394 **Conclusions**

395 This study highlighted the presence of clinical signs and behavioral manifestations which can
396 be interpreted as expression of pain in amputated cats.

397 Furthermore, the presence of behavioral manifestations and alterations in daily habits in the
398 pre-amputation period resulted to be related to the presence of pain in this period, while the
399 time elapsed between diagnosis and amputation was significantly correlated to the
400 development of pain in the post-amputation period. Finally, the measurement of the
401 mechanical nociceptive threshold at the level of the amputated region highlighted a mean
402 nociceptive threshold in the affected area significantly lower than the healthy contralateral
403 dermatome, confirming a development of pathologic pain perception over time probably due
404 to a reorganization of the peripheral/central sensory pathways.

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407 *References*

408 Collins, K. L., Russel, H. G., Schumacher, P. J., Robinson-Freeman, K. E., O’Conor, E. C.,
409 Gibney, K. D., Yambem, O., Dykes, R. W., Waters, R. S., Tsao, J. W., 2018. A review of
410 current theories and treatments for phantom limb pain. *J. Clin. Invest.* 128, 2168-2176.

411 Dahm, P. O., Nitescu, P. V., Appelgren, L. K., Curelaru, I. D., 1998. Long-term intrathecal
412 infusion of opioid and/or bupivacaine in the prophylaxis and treatment of phantom limb pain.
413 *Neuromodulation* 1, 111- 128.

414 Della Rocca, G., Di Salvo, A., Vuerich, M., Mandara, M. T., 2011. Il dolore neuropatico
415 negli animali. *Veterinaria* 25, 7-22.

416 Dickerson, V.M., Coleman, K.D., Ogawa, M., Saba, C.F., Cornell, K.K., Radlinsky, M.G.,
417 Schmiedt, C.W., 2015. Outcomes of dogs undergoing limb amputation, owner satisfaction
418 with limb amputation procedures, and owner perceptions regarding post- surgical adaptation:
419 64 cases (2005-2012). *J. Am. Vet. Med. Assoc.* 247, 786-792.

- 420 Di Giminiani, P., Edwards, S.A., Malcolm, E.M., Leach, M.C., Herskin, M.S., Sandercock,
421 D.A., 2017. Characterization of short- and long-term mechanical sensitisation following
422 surgical tail amputation in pigs. *Sci. Rep.* 7, 4827.
- 423
- 424 Dixon, M.J., Taylor, P.M., Steagall, P.V.M., Brondani, J.T., Luna, S.P.L., 2007.
425 Development of a pressure nociceptive threshold testing device for evaluation of analgesics
426 in cats. *Res. Vet. Sci.* 82, 85–92.
- 427
- 428 Flor, H., 2002. Phantom-limb pain: characteristics, causes and treatment. *Lancet Neurol* 1,
429 182-189.
- 430 Fischer, A.A., 1998. Algometry in diagnosis of musculoskeletal pain and evaluation of
431 treatment outcome: an update. *J. Musculoskelet. Pain* 6, 5-32.
- 432 Forster, L. M., Wathes, C. M., Bessant, C., Corr, S. A., 2010. Owners' observations of
433 domestic cats after limb amputation. *Vet. Rec.* 167, 734- 739.
- 434 Galindo-Zamora, V., von Babo, V., Eberle, N., Betz, D., Nolte, L., Wefstaedt, P., 2016.
435 Kinetic, kinematic, magnetic resonance and owner evaluation of dogs before and after the
436 amputation of a hind limb. *BMC Vet. Res.* 12, 20.
- 437 Hanley, M.A., Jensen, M.P., Smith, D.G., Ehde, D.M., Edwards, W.T., Robinson, L.R., 2007.
438 Pre-amputation pain and acute pain predict chronic pain after lower extremity amputation. *J.*
439 *Pain* 8, 102-109.
- 440 Harris, L.K., Whay, H.R., Murrell, J.C., 2018. An investigation of mechanical nociceptive
441 thresholds in dogs with hind limb joint pain compared to healthy control dogs. *Vet J* 234, 85-
442 90.

- 443 Hill, A., 1999. Phantom limb pain: a review of the literature on attributes and potential
444 mechanisms. *J. Pain. Symptom Manage* 17, 125-142.
- 445 Holden, E., Calvo, G., Collins, M., Bell, A., Reid, J., Scott, E. M., Nolan, A. M., 2014.
446 Evaluation of facial expression in acute pain in cats. *J. Small Anim. Pract.* 55, 615-621.
- 447 Hui, B., Hughes, D., Wu, H., Bhatti, O., Zhao, S., Johnson, M., 2012. An MR safe algometer
448 to study phantom and residual limb pain. *Annu Int Conf IEEE Eng Med Biol Soc.* 2012, 146-
449 149.
- 450 Hunt, J., Knazovicky, D., Lascelles, B.D.X., Murrell, J., 2019. Quantitative sensory testing in
451 dogs with painful disease: A window to pain mechanisms?. *Vet. J.* 243, 33–41
- 452 Kirpensteijn, J., van den Bos, R., Endenburg, N., 1999. Adaptation of dogs to the amputation
453 of a limb and their owners' satisfaction with the procedure. *Vet. Rec.* 144, 115-118.
- 454 Lambert, A. W., Dashfield, A. K., Cosgrove, C., Wilkins, D. C., Walker, A. J., Ashley, S.,
455 2001. Randomized prospective study comparing preoperative epidural and intraoperative
456 perineural analgesia for the prevention of postoperative stump and phantom limb pain
457 following major amputation. *Re. Anesth. Pain Med.* 26, 316-321.
- 458 Luo, Y., Anderson, T. A., 2016. Phantom limb pain: a review. *Int. Anesthesiol. Clin.* 54, 121-
459 139.
- 460 Melzack, R., 1971. Phantom limb pain: implications for treatment of pathologic pain.
461 *Anesthesiology* 35, 409-419.
- 462 Mathews, K., Kronen, P.W., Lascelles, D., Nolan, A., Robertson, S., Steagall, P.V.M.,
463 Wright, B., Yamashita, K., 2014. Guidelines for recognition, assessment and treatment of

- 464 pain. *J. Small Anim. Pract.* 55, E10-E68
- 465
- 466 Menchetti, M., Gandini, G., Gallucci, A., Della Rocca, G., Matiasek, L., Matiasek, K.,
467 Gentilini, F., Rosati, M., 2017. Approaching phantom complex after limb amputation in the
468 canine species. *J. Vet. Behav.* 22, 24-28.
- 469 Merola, I., Mills, D. S., 2016. Behavioural signs of pain in cats: an expert consensus. *PloS*
470 *one* 11, e0150040.
- 471 Nikolajsen, L., Jensen, T. S., 2000. Phantom limb pain. *Curr. Rev. Pain.* 4, 166-170.
- 472 Nikolajsen, L., Jensen, T.S., 2001. Phantom limb pain. *Br. J. Anaesth.* 87, 107-116.
- 473 Nikolajsen^a, L., Ilkjaer, S., Kroner, K., Christensen, J.H., Jensen, T.S., 1997. The influence of
474 preamputation pain on postamputation stump and phantom pain. *Pain* 72, 393-405.
- 475 Nikolajsen^b, L., Ilkjaer, S., Christensen, J. H., Krøner, K., Jensen, T. S., 1997. Randomised
476 trial of epidural bupivacaine and morphine in prevention of stump and phantom pain in
477 lower-limb amputation. *Lancet* 350, 1353-1357.
- 478 Pell, J. P., Donnan, P. T., Fowkes, F. G. R., Ruckley, C. V., 1993. Quality of life following
479 lower limb amputation for peripheral arterial disease. *Eur. J. Vasc. Surg.* 7, 448-451.
- 480 Probstner, D., Thuler, L.C., Ishikawa, N.M., Alvarenga, R.M., 2010. Phantom limb
481 phenomena in cancer amputees. *Pain Pract.* 10, 249-256.
- 482 Rolke, R., Magerl, W., Campbell, K. A., Schalber, C., Caspari, S., Birklein, F., Treede, R. D.,
483 2006. Quantitative sensory testing: a comprehensive protocol for clinical trials. *Eur. J. Pain*
484 10, 77-88.

- 485 Sinha, R., van den Heuvel, W. J., Arokiasamy, P., 2011. Factors affecting quality of life in
486 lower limb amputees. *Prosthet. Orthot. Int.* 35, 90-96.
- 487 Taylor, P.M., Robertson, S.A., Dixon, M., 2007. Evaluation of the use of thermal thresholds
488 to investigate NSAID analgesia in a model of inflammatory pain in cats. *J. Feline Med. Surg.*
489 9, 313-318.
- 490 Troncoso, R.J., Herzberg, D.E., Meneses, C.S., Müller, H.Y., Werner, M.P., Bustamante, H.,
491 2018. Mechanical/thermal sensitivity and superficial temperature in the stump of long-term
492 tail-docked dairy cows. *PeerJ.* 6, e5213.
- 493 Waran, N., Best, L., Williams, V., Salinsky, J., Dale, A., Clarke, N., 2007. A preliminary
494 study of behaviour-based indicators of pain in cats. *Anim. Welfare* 16, 105-108.
- 495 Withrow, S.J., Hirsch, V.M., 1979. Owner response to amputation of a pet's leg. *Vet. Med.*
496 *Small. Anim. Clin.* 74, 332-334.

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499 ***Supplementary Tables***

500

	Yes	No
Pain before amputation	17/27 (63%)	6/27 (37%)
Pain after amputation	23/27 (85%)	4/27 (15%)

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502 **Supplementary Table 1: pain behaviors reported by the WSAVA guidelines, the**
503 **majority of owners described several indicators of pain or discomfort both before and**
504 **after amputation.**

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Muscular twitching in the stump region	11/13 (85%)
Licking the stump	5/13 (38%)
Looking at the stump	5/13 (38%)
Restlessness	4/13 (31%)
Preferring to lie on a chilly floor	3/13 (23%)
Looking anxious	2/13 (15%)
Attitude of isolation	2/13 (15%)
Continuous change of position to find comfort	2/13 (15%)
Reluctance to move	1/13 (8%)
Biting and/or scratching the affected limb	1/13 (8%)
Vocalization	1/13 (8%)
Low ears	1/13 (8%)
Contracted cheeks	1/13 (8%)
Aggression toward animals	1/13 (8%)
Aggression toward humans	1/13 (8%)

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Supplementary table 2: pain behaviors described by the owners in the time frame comprised between 1 month and more than 1 year after amputation.

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