Approaching phantom complex after limb amputation in cats

Abstract

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

This pilot study introduces previously unreported signs that may be interpreted as expressions of pain in amputee cats.

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

Introduction

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

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

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

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

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

(Withrow and Hirsch, 1979; Kirpensteijn, van den Bos, and Endenburg, 1999; Dickerson et al., 2015; Galindo-Zamora et al., 2016). A recent study described the occurrence of PLC signs in dogs undergoing limb amputation. In particular, the duration of pre- amputation pain and time between diagnosis and amputation were identified as risk factors for a higher frequency of post-amputation pain episodes in amputated dogs (Menchetti et al., 2017). Post-amputation limb behavior changes have been described in amputated cats (Forster et al., 2010). However, aspects relating to the onset and semiology of these changes have not yet been investigated. Despite a lack of evaluation scales for neuropathic pain in animals, direct measurements for the investigation of thermal and mechanical nociceptive threshold have been published (Dixon et al., 2007; Taylor et al, 2007; Harris et al., 2018; Hunt et al., 2019). Whit specific reference to amputation, the measurement of mechanical nociceptive threshold for the evaluation of post-amputation sensitization following tail docking in cow and swine has been successfully applied (Di Gimignani et al., 2017; Troncoso et al., 2018).

The aims of the present study were to document the prevalence of PLC in a population of cats with limb amputation, identifying signs and behaviors suggestive of neuropathic pain, evaluate risk factors associated with PLC occurrence and determine the owners' perceptions of the quality of life (QoL) of their 3-legged pets. Secondly, measurement of mechanical nociceptive threshold was implemented in the clinical evaluation of selected patients for objective identification of allodynia and/or hyperalgesia.

Materials and Methods

Study population

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

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

Questionnaire design and description

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

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

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

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

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

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

The questionnaire was administered to the owners by phone interview during February 2018. Ethical approval was granted by the University of Bologna ethics committee (ID 664/2016). An English translation of the original Italian version is available as supplementary material.

Mechanical nociceptive threshold assessment

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

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

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

Statistical analysis

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

Results

Descriptive data

A total of 27 cats that underwent limb amputation were included in the study. All owners (27/27) answered the questionnaire, while the MNT assessment was conducted in 12/27 (44%) patients. At the time of the questionnaire, the majority of cats included in the study were still alive (17/27; 63%).

All cats were European Shorthair (ESH) breed. Seventeen (63%) were males, 13 of which (76%) neutered, whereas 10 (37%) were females, 5 of which (50%) spayed. At the time of amputation, the median age was 5 years (range 4 months - 18 years) and the mean weight was 3.98 kg (± 0.35).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Pain related manifestations and behavioral changes

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

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 cheecks	1/13 (8%)
Aggression toward animals	1/13 (8%)
Aggression toward humans	1/13 (8%)

Table 1: pain behaviors described by the owners in the timeframe comprised between 1 month and more than 1 year after amputation.

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

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

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%)

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

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

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

Accessory symptom that could possibly account for pain, were described by 9/27 owners (33%) in the pre-amputation period and 5/27 owners (19%) in the post-amputation period. Environmental factors and/or physical stress, as judged by the owner, were reported in relation to the onset of pain in 4/27 cats (15%).

Post-surgical complications

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

Therapies

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

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

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

When specifically asked about drugs administered because of pain after amputation, the most frequent reported medications were anti-inflammatory drugs (14/27) followed by pain killers (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

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

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

Discussion

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

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

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

Despite the majority of owners in our study described the presence of pain during the first week after surgery, a small number reported its persistence for more than one year. This 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 folllowing 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

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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

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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%)

501
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 cheecks	1/13 (8%)
Aggression toward animals	1/13 (8%)
Aggression toward humans	1/13 (8%)

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