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Long-term hyperalgesia and traumatic neuroma formation in tail-docked lambs

C Larrondo*[†], H Bustamante[‡], E Paredes[§] and C Gallo[#]

[†] Escuela de Graduados, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Independencia 631, Valdivia, Chile
[‡] Instituto de Ciencias Clínicas Veterinarias, Universidad Austral de Chile, Independencia 631, Valdivia, Chile
[§] Instituto de Patología Animal, Universidad Austral de Chile, Independencia 631, Valdivia, Chile
[#] Instituto de Ciencia Animal, OIE Collaborating Centre for Animal Welfare and Livestock Production Systems-Chile, Universidad Austral de Chile, Independencia 631 Valdivia, Chile
* Contact for correspondence: cristian.larrondoc@gmail.com

Abstract

This study aimed to determine if tail-docking induces long-term hyperalgesia, chronic pain and histopathological changes in tail stumps of tail-docked lambs. Fifty male lambs of 45 days of age were randomly allocated in two groups. One group of 25 lambs was tail-docked using a hot cautery iron and a second group of 25 lambs was subjected only to handling as a control group (undocked lambs). Prior to tail-docking and at intervals of 15, 30, 60 and 90 days after the procedure, infra-red thermography (IT) and mechanical nociceptive thresholds (MNTs) tests were carried out in both lambs' tails/stumps, and animals were weighed. In addition, the visual degree of inflammation of tail stumps was evaluated. Finally, animals were slaughtered in a commercial slaughterhouse and tail sections of ten lambs from each group were examined histopathologically. Tail-docking was associated with an inflammatory process according to IT and visual observation in tail stumps at 15 and 30 days post-docking. Tail-docked lambs had lower MNTs than undocked lambs at all evaluated times after tail-docking, indicating the presence of long-term hyperalgesia. Also, traumatic neuroma formation was found in tail stumps of 2/10 tail-docked lambs, and 6/10 presented neuromatous tissue development. It is concluded that tail-docking induces acute and chronic pain in lambs, initially through inflammation, and then via long-term hyperalgesia and traumatic neuroma formation. These long-term findings would have negative implications for the animal welfare of tail-docked lambs.

Keywords: *animal welfare, chronic pain, hyperalgesia, lamb, neuroma, tail-docking*

Introduction

Tail-docking is a routine practice in most sheep-producing countries (Grant 2004; Zanolini 2006; Fisher *et al* 2007). The main productive and sanitary justification is the improvement of the sanitary conditions of the herds as a result of removing a portion of the tail of lambs, because there would be less accumulation of faeces and urine in the tail and breech area, thus decreasing the incidence of flystrike (Scobie & O'Connell 2002; Grant 2004; Zanolini 2006; Clark *et al* 2011). Under this premise, tail-docking would be a practice that would improve animal welfare and health in ovine flocks (Fisher *et al* 2007). However, currently, there is no consensus in the scientific field regarding the implications from the point of view of the welfare and health of the animals subjected to this handling (Fisher *et al* 2007; Fisher & Gregory 2007; Sutherland & Tucker 2011).

It has been demonstrated that tail-docking in lambs, irrespective of the technique, ie whether surgically, with a knife, hot iron or rubber ring, produces acute pain in the animals, affecting their physiology, anatomy, and welfare (Grant 2004; Sutherland & Tucker 2011). However, there is considerable evidence supporting the hypothesis that tail-docking could generate chronic pain in animals, which at present has not been well characterised (Viñuela-Fernández *et al* 2007; Di Giminiani *et al* 2017). In humans, the presence of neuropathic pain is described in 60–80% of people who have undergone the amputation of a limb (Viñuela-Fernández *et al* 2007; Nikolajsen 2012). Neuropathic pain originates as a consequence of an injury or illness of the somatosensory system and may persist for some time after the acute phase of injury, transforming itself into chronic pain (Devor *et al* 2014).

Currently, there is an increasing interest, from a research and animal welfare standpoint, on the routine procedures of productive species that have the potential to cause chronic pain in animals (Viñuela-Fernández *et al* 2007). Pathologies and inflammatory processes are some of the main sources of pain in ruminants (Fitzpatrick *et al* 2006). A tool that has been validated in various species for the evaluation of animal welfare and inflammatory processes is infra-red thermography (IT) (Stewart *et al* 2009; Dowling *et al* 2013; Rekant *et al* 2016; Van der Saag *et al* 2018). In this sense, IT can be used to determine local inflammation generated as a consequence of productive procedures that involve an increase of temperature, such as marking, dehorning and castration (Stewart *et al* 2008, 2009; Dowling *et al* 2013; Van der Saag *et al* 2018).

The inflammatory component involved in painful procedures induces alterations in the processing of nociceptive information, such as hyperalgesia, which is defined as the exaggerated response to a noxious stimulus (Zimmermann 2001; Manteca *et al* 2017). Hyperalgesia has been used in various species as an indirect indicator of pain, allowing also to relate the severity of an inflammatory process over time (Fitzpatrick *et al* 2006). It is described that hyperalgesia, as a consequence of an inflammatory condition, is associated in the first instance with spontaneous pain, leading to reduced well-being for a longer period of time (Fitzpatrick *et al* 2006).

A widely used test to evaluate hyperalgesia in animals is the determination of mechanical nociceptive thresholds (MNTs) through the use of an algometer (Fitzpatrick *et al* 2006; Love *et al* 2011; Janczak *et al* 2012). In the case of mice and pigs, after tail-docking the animals show a decrease in the MNTs on the tail remnant, both in the short and long term, in contrast with undocked animals (Zhuo 1998; Di Giminiani *et al* 2017). However, it is unknown if lambs subjected to tail-docking experience chronic pain and changes at the MNT level (French & Morgan 1992; Viñuela-Fernández *et al* 2007), as well as whether there is a relationship between the inflammatory process and pain after the procedure.

Long-term hyperalgesia in tail-docked animals has been associated with neuropathic pain and the presence of neuromas (Zimmerman 2001; Eicher *et al* 2006; Toia *et al* 2015; Sandercock *et al* 2016). Neuromas are non-neoplastic proliferations of nervous tissue, which can induce nervous excitability changes, central and/or peripheral sensitisation and ectopic impulse discharge, mainly in C-fibres (French & Morgan 1992; Zimmermann 2001; Devor *et al* 2014; Okafor *et al* 2014). These and other changes possibly lead to the occurrence of neuropathic pain, which can be chronic (Devor *et al* 2014), in addition to an exaggerated behavioural response of the animals to local thermal and mechanical stimulation tests (Zimmermann 2001; Eicher *et al* 2006; Okafor *et al* 2014; Sandercock *et al* 2016).

The presence of neuromas in tail-docked animals, can cause pain and discomfort even once the tail stump is fully healthy and healed. These alterations may persist in animals for weeks or even years after tail-docking (Farm Animal Welfare Council [FAWC] 2008; Animal Welfare Approval [AWA] 2009). Although, it is not known if there is a relationship between the inflammatory process and pain in time after tail-docking. In addition, it is unknown if tail-docked lambs experience nociceptive threshold changes, chronic pain, and neuroma formation after tail-docking. The objective of the present study was to determine the existence of changes in temperature and inflammation in the stump of tail-docked lambs, as well as in the MNTs, from tail-docking to slaughter and neuroma formation in tail stumps.

Materials and methods

This study was approved by the Committee of Bioethics and Use of Animals in Research of the Universidad Austral de Chile (Nº 288/2017).

Location of study

The study was conducted between September and December 2017 at Austral Agricultural Experimental Station, owned by the Universidad Austral de Chile. The property is located in the city of Valdivia, Los Ríos Region, Chile.

Study animals and management

Fifty male lambs of 45 days of age and weighing a (mean ± SD) of 18.08 (± 4.18) kg were used, and the animals randomly allocated to two groups. No power analysis was carried out, but the sample size used in our research was similar to other studies using a mechanical nociceptive test (Stubsjøen *et al* 2010; Di Giminiani *et al* 2015). A first group was made up of 25 animals that underwent tail-docking (tail-docked lambs) at the level of the third intercoccygeal space by means of a hot cautery iron (Te Pari Docking Iron®, Standard Model, Te Pari, New Zealand) while the second was composed of 25 lambs subjected only to handling, as a control (undocked lambs). Prior to tail-docking, local anaesthesia (2 ml lidocaine 2% with 23-G needle) was applied dorsally and laterally at the area of the point of docking (only to tail-docked lambs). Although international recommendations suggest performing tail-docking at an early age (AWA 2009; Sutherland & Tucker 2011), in the present study it was carried out at the above mentioned age, since it is within the age range for this practice in most of the extensive systems of ovine breeding (Sutherland & Tucker 2011). Experimental station personnel monitored behavioural indicators that could be indicative of fever and/or pain associated with docking daily, including lack of appetite, ataxia, separation from the flock, depression and weight loss. If one or more animals presented any of these signs, personnel had to immediately inform the first author, in order to inspect affected animals. In circumstances such as presence of fever and/or severe pain, due to infection after docking, the protocol stated that the lamb was to be treated accordingly and, if the problem persisted, removed from the experiment altogether. However, this never proved to be the case.

Description of the experimental work

The management and tests throughout the study were carried out by the same, trained researcher, in a pen inside a shed with cement floor and natural light. Animals were kept in the same paddock without isolation from the rest of the flock. Prior to tail-docking (time 0, basal sampling), the following tests were carried out in the tail of tail-docked and undocked lambs: IT with a thermal camera (FLIRi5, FLIR Systems, Wilsonville, OR, USA) by means of a dorsal view, and evaluation of the mechanical nociceptive thresholds (MNTs) with a portable algometer (Wagner Force Ten FDX 25 Compact Digital ForceGage, Wagner Instruments, Riverside, CT, USA). Then, the animals were identified by numbered ear-tags with a unique number and weighed with

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a Digital Roman Scale (Pratlley®, 3-way Swing Gate AutoDraft, New Zealand).

To record the thermographic images and the mechanical pressure tests, the animals were manually immobilised, in order to limit stressing them. Lambs were kept within a small pen during the measurements, so they could be close enough for easy catching but far enough away to not touch others when immobilised, in order to properly visualise behavioural reactions. One-by-one, they were immobilised by one trained farm animal handler who held the lamb gently between the legs while standing on the floor and placed a hand below the lamb's chin. The evaluation of the MNTs was carried out after the IT, once the animals were calm, without separating them from the rest of the study flock, thereby avoiding stress and escape attempts due to isolation. Once the tests were completed, the animals were released from the pen and reunited with their dams.

Subsequent to tail-docking and at intervals of 15, 30, 60 and 90 days, the above-mentioned tests and the weighing of lambs were repeated, adding to the IT a central view of the tail stumps in docked lambs. In addition, signs of tail-stump inflammation in tail-docked lambs were noted down and a picture of each stump was taken. The data lead to the following inflammation degree scale: 0) absent: there are no signs of inflammation in the tail stump; 1) slight: slight swelling and flushing; 2) moderate: moderate swelling, flushing, tissue turgid to the touch; and 3) intense: intense swelling, flushing, tissue turgid to the touch, pain to the touch, there may be exudate and/or blood. At the end of the study, intra-observer reliability regarding degree of inflammation was assessed by scoring all pictures of inflamed tail stumps twice. Intra-observer agreement was good (Cohen's kappa = 0.90; $P < 0.05$).

Infra-red thermography

The IT of the tail in both groups of lambs (undocked/docked, dorsal view) and central tail stump view of tail-docked lambs, was performed from a distance of 0.5 m. The thermograms were stored in JPEG format and later analysed using FLIR Tools 3.1 software (FLIR Systems, Wilsonville, OR, USA). The thermographic records were corrected in the software by the atmospheric temperature (ºC) and relative humidity (%); these data were recorded at each sampling point with a digital recorder (ElitechURC-4HC®, Elitech, USA). In order to analyse the thermographic images for the dorsal view of the tail of both groups, an area from the base of the tail to the stump (docked lambs) was selected, and at a length equivalent to the tail of undocked animals (average length: 8 cm). For the dorsal view, three parallel lines were drawn in each image using the software, to later obtain an average of the three points. To analyse the central view of the tail stump, an oval area was created and the average temperature of the area calculated.

Evaluation of the mechanical nociceptive thresholds

Lambs were immobilised manually and the individual taking the measurements gently held the tail with one hand, holding it from underneath without pressure while, with the other, applied dorsal and perpendicular pressure to the tail stump of docked lambs, no more than 1 cm from the site of the docking. In the undocked lambs, pressure was applied with the algometer at equivalent length of the tail. The researcher had been trained in the use of the portable algometer and applied pressure with the tip (1 cm^2) at an approximate speed of 5 Newtons (N) of force per second, carrying out two measurements, two minutes apart, which were then averaged to create a single value. The following reflex movements were considered as a positive response to the test: i) lateral movement of the tail, defined as one or more lateral shakes of the tail; ii) arching of the back, where the animal arches its column; and iii) kick, when the animal kicks the ground with a hind limb or extends it without hitting the ground. To induce a painful stimulus, the maximum applied mechanical pressure limit was 75 N (Lomax *et al* 2010), allowing the manual algometer to visually assess that the stimulus did not cause tissue damage (Stubsjøen *et al* 2010). Observations were made at the same time as the tests by the same trained researcher. The behaviours used were validated and based on a previous pilot study as well as previous studies (Lomax *et al* 2010). Intra-observer agreement was not possible to obtain but observations and recording were carried out by the same veterinarian and behaviours had been previously observed in a previous pilot study.

Histopathology

Following the study, ten lambs of each group (ten undocked and ten docked; n = 20) were randomly selected and slaughtered in a commercial slaughterhouse. In tail-docked lambs, tail stumps of 3 cm from the tip were cut off with a scalpel. Similar tail samples in length and anatomical location were obtained from undocked lambs. Samples were fixed in 10% formalin for 15 days, then transferred to 1% nitric acid for 15 days for decalcification. Samples were processed according to a laboratory protocol, through graded alcohols and xylene, embedded in paraffin, cross-sectioned at 5-6 μm and stained with haematoxylin and eosin. In addition, similar sections of the same tissue samples were stained with Masson's trichrome, to optimise the visualisation and description of the epineurial, perineurial and endoneurial connective tissue.

Tissue samples were examined using an optical microscope (Olympus CX31, Olympus, Japan) at 4, 10 and 40× magnification. Histopathological description was made regarding the degree of epineurial, perineurial and endoneurial connective tissue fibrosis, as well as proliferation of the nervous tissue and angiogenesis.

Statistical analysis

Data were analysed with the statistical programme SPSS version 22.0 (SPSS Inc, Chicago, IL, USA) with lamb as the experimental unit. To evaluate the existence of tail IT differences (dorsal view) between the treatment groups (undocked/docked) over time, a mixed linear model with repeated measures was used, with the sampling day, interaction between the day of sampling and treatment groups, and live weight (kg) as fixed effects, and the 'animal' as a random effect. A similar mixed linear model was used with the infra-red temperature (central view) of the stump of tail-docked lambs, but only including the effect of time. The same mixed linear model was used for MNTs of the tail. Spearman correlations were performed ($P < 0.05$) between the MNTs and IT of tail-docked lambs. In addition, the effect of the degree of stump inflammation of tail-docked lambs on the MNTs was analysed by a linear model, where the degree of inflammation and the day of sampling were the fixed effects (with two classes; 15 and 30 days after tail-docking). The variable live weight was not significant ($P > 0.05$) on the variable MNTs, therefore it was removed from the final statistical model.

Histopathological findings were described according to each sample's relative abundance (proliferation of nerve bundles and fibrosis of epineurial, perineurial and endoneurial tissue, in contrast to the control): – absent; + low; ++ moderate; +++ high/marked. According to these histopathological findings, samples were classified on a scale, similar to that used by Kells *et al* (2016), but including category 0 as normal, where: 0) normal morphology of nerve bundles, without proliferation and fibrosis of epineurial, perineurial and endoneurial tissue; 1) mild fibrosis of dense fibrous connective tissue in epineurial, perineurial and endoneurial tissue, without disorganised proliferation of nerve bundles; 2) moderate increase of disorganised nerve bundles, surrounded by moderate fibrosis of dense fibrous connective tissue in epineurial, perineurial and endoneurial tissue; and 3) marked increase of disorganised nerve bundles of different sizes, surrounded by fibrosis of dense fibrous connective tissue in epineurial, perineurial and endoneurial tissue. The presence of abnormal proliferation of nerve bundles, plus fibrosis of dense fibrous connective tissue in epineurial, perineurial and endoneurial tissue (score 2 and 3), were indicative of neuroma formation (Kells *et al* 2016).

Results

Infra-red thermography

When evaluating tail IT (dorsal view) between tail-docked and undocked lambs 15, 30, 60 and 90 days after tail-docking, no significant differences ($P > 0.05$) were found in the temperature between both groups, at each time of sampling (Figure 1). Similarly, there was no interaction ($P > 0.05$) between IT and the sampling day.

The infra-red temperature of the tail stumps from tail-docked lambs experienced a significant decrease between 15 vs 30, and 30 vs 60 days ($P < 0.05$). Fifteen days after tail-docking, the highest average temperature was recorded (36.25 [± 2.88]ºC), a value that decreased progressively until 32.04 [± 1.45]ºC at 90 days. Between 60 and 90 days after tail-docking, there were no significant differences ($P > 0.05$) in the IT of the tail stumps (Figure 2).

Mechanical nociceptive thresholds

A total of 255 mechanical pressure tests were recorded during the study; 5.5% of the tests (14/255) being above the threshold of 75 N (maximum applied mechanical pressure limit) and corresponding to undocked lambs in different sampling points. These data were not considered in the statistical analysis.

A significant difference ($P < 0.05$) was found in the MNTs between groups (undocked/tail-docked) at all the evaluated times (15, 30, 60 and 90 days) after tail-docking (Figure 3). At day 15, tail-docked lambs presented the lowest MNTs compared to undocked lambs (9.84 [± 5.61] and 50.98 [± 11.68] N, respectively), whereas at 90 days this difference was smaller (30.97 [± 8.07] and 51.15 [± 9.43] N) but still significant ($P < 0.05$). In addition, there was a significant interaction ($P < 0.05$) between the MNTs and the sampling time, evidencing an increase in the MNTs of tail-docked lambs with time (Figure 3).

Degree of inflammation in the stump of tail-docked lambs

Ninety-six percent of tail-docked animals (24/25), presented with inflammation of the stump (grade 1 to 3) at day 15 after tail-docking. At this sampling, more than half of the animals (60%; 15/25) were found with inflammation classified as intense (grade 3). In contrast, 30 days after the procedure, 44% (11/25) showed intense inflammation, while 20% of the lambs (5/25) had no inflammation (grade 0). In the subsequent samplings (60 and 90 days), there were no signs of inflammation in the stump.

The degree of inflammation of the stump at 15 and 30 days after tail-docking had a significant effect ($P < 0.05$) on the MNTs (Figure 4). The animals that did not show any visual signs of inflammation (grade 0), showed higher MNTs than those with moderate and intense degrees of inflammation (2 and 3) in both sampling times. There were no differences ($P > 0.05$) in the MNTs between the degrees of inflammation 1 and 2, in both sampling points. The interaction between the degree of inflammation and the sampling time was not significant ($P > 0.05$). In addition, a negative and significant correlation was found ($rho = -0.51$; $P = 0.0001$) between the MNTs and the degree of inflammation. The degree of inflammation did not have an effect on the IT of the stump ($P > 0.05$).

Relationship between the MNTs and IT of the stump of tail-docked lambs

There was a negative and significant correlation ($r = -0.47$; $P < 0.05$) between the MNTs and the IT of the stump of tail-docked lambs (Figure 5). Fifteen and 30 days after tail-docking, docked lambs presented the highest values of temperature in their stumps, and at the same time the lowest MNTs, in contrast to later sampling times. The IT of the stump diminished with the course of time, while the values of the MNTs increased.

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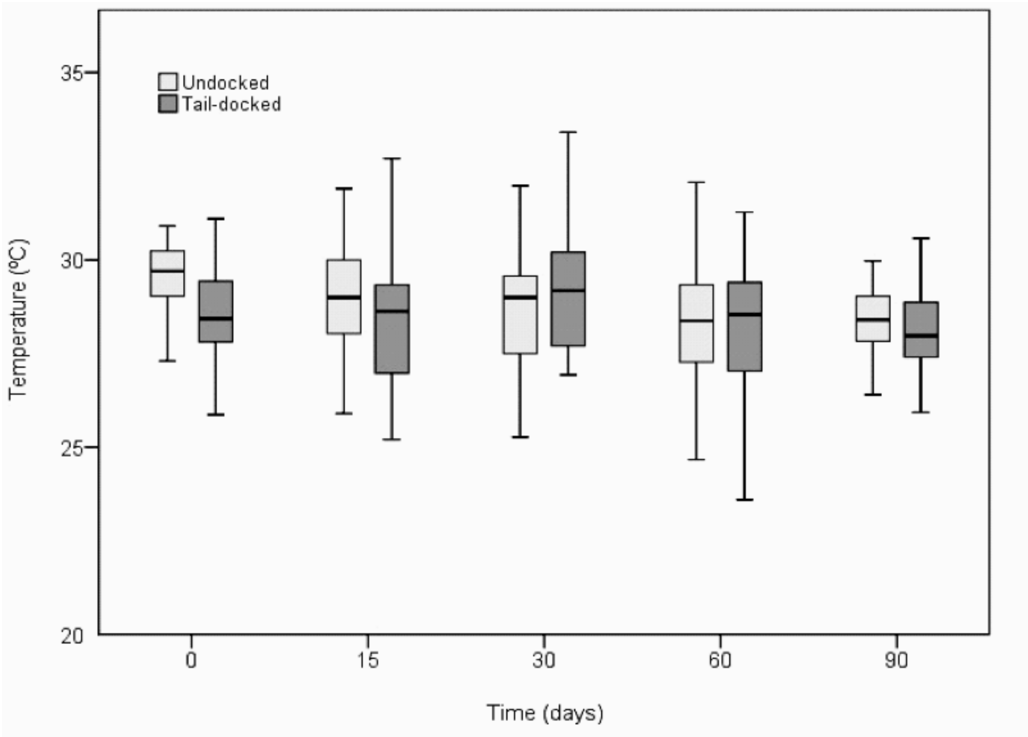
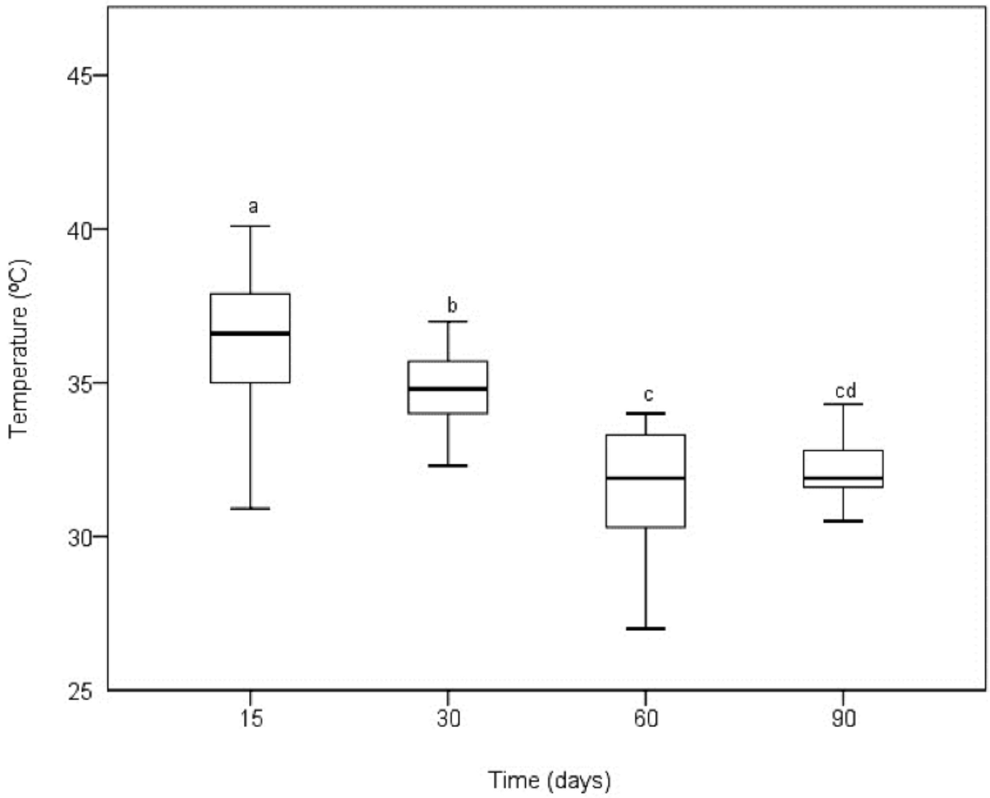
Figure 1



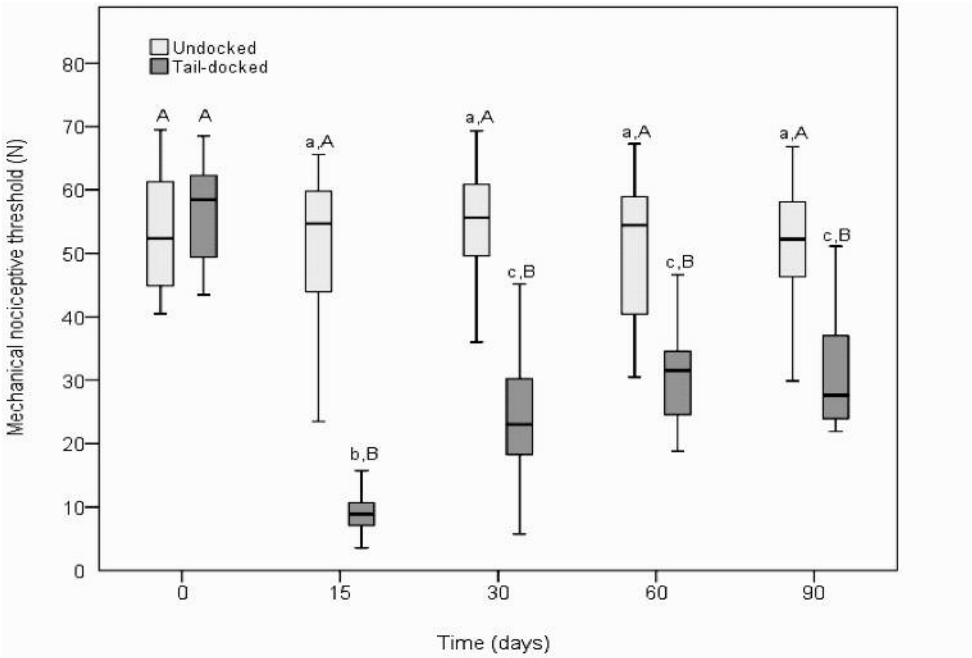
Box and whisker plot of infra-red temperature (°C) of the tail (dorsal view) in undocked vs tail-docked lambs, before tail-docking (0), and 15, 30, 60 and 90 days after the procedure.

Figure 2



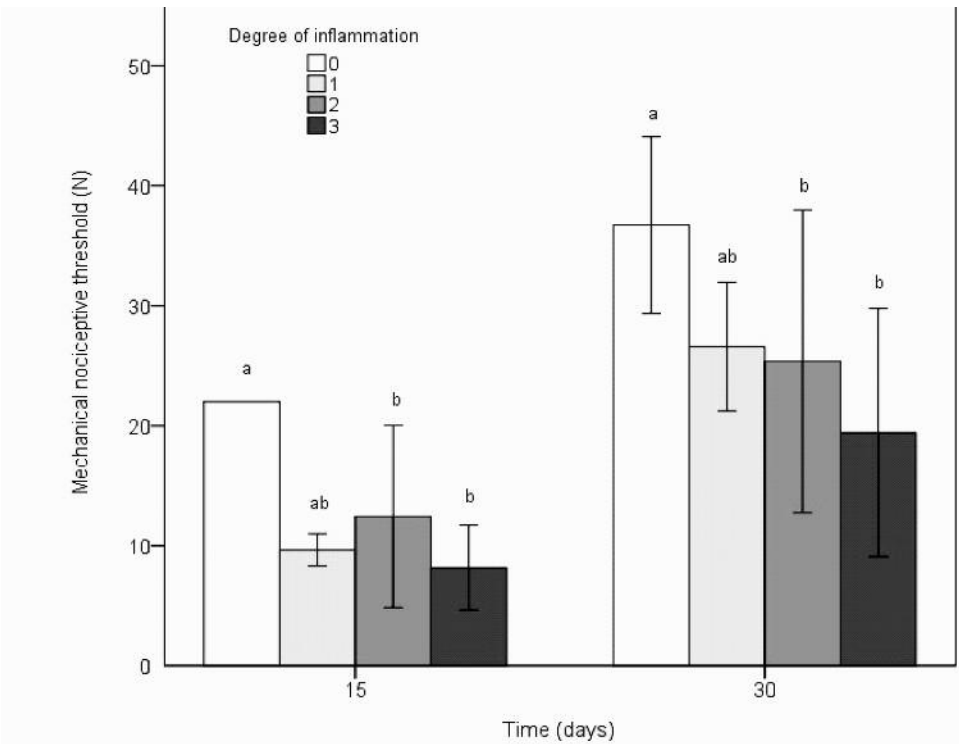
Box and whisker plot of infra-red temperature (°C) in the stump of tail-docked lambs, 15, 30, 60 and 90 days after tail-docking. Different superscripts indicate significant differences ($P < 0.05$).

Figure 3



Box and whisker plot of mechanical nociceptive thresholds (N) in different sampling times: previous to tail-docking (0), 15, 30, 60 and 90 days after the procedure, in undocked and tail-docked lambs. Different lowercase letters represent significant differences ($P < 0.05$) within each group with the previous sampling time, while different uppercase letters represent significant differences between groups in each sampling time.

Figure 4



Mean (± SD) mechanical nociceptive thresholds, according to inflammation degree scale of tail stump of tail-docked lambs, 15 and 30 days after the procedure. Score 0 = Absent, there are no signs of inflammation in the tail stump; Score 1 = Slight, slight swelling and flushing; Score 2 = Moderate, moderate swelling, flushing, tissue turgid to the touch; score 3 = Intense, intense swelling, flushing, tissue turgid to the touch, pain to the touch, there may be exudate and/or blood. Different supercripts for each sampling day indicate significant differences ($P < 0.05$).

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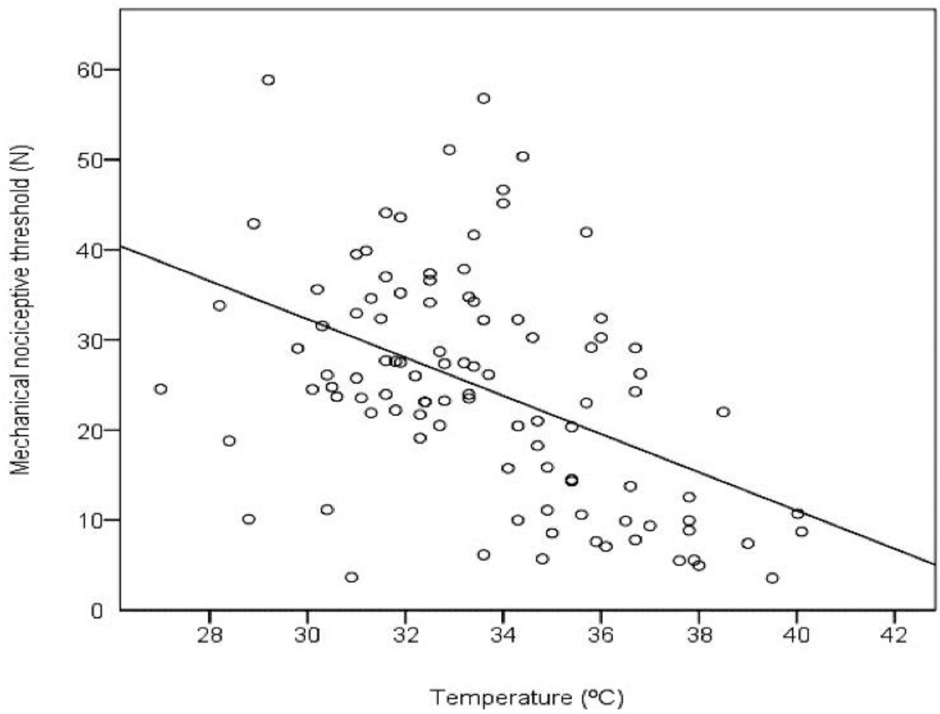
Figure 5



Relationship between the mechanical nociceptive thresholds (N) and infra-red temperature (°C) of the tail stumps of tail-docked lambs. $r = -0.47$; $P < 0.05$.

Histopathological findings

All the control samples (n = 10) were classified with score 0 (Figure 6). In 70% (7/10) of tail-docked samples, moderate to marked fibrosis of the epineurial and perineurial connective tissue was observed, while the remaining 30% only had mild fibrosis. Angiogenesis was present in all of these samples, with the majority (80%; 8/10) classified as mild. Regarding the organisation and the presence of proliferation of nerve bundles, 60% of samples had mild peripheral nerve proliferation, while 20% had a marked and disorganised proliferation. Considering these histopathological findings, the classification of tail-docked samples was: score 0 = 20%; score 1 = 60%; score 2 = 0%; score 3 = 20% (Figures 7 and 8).

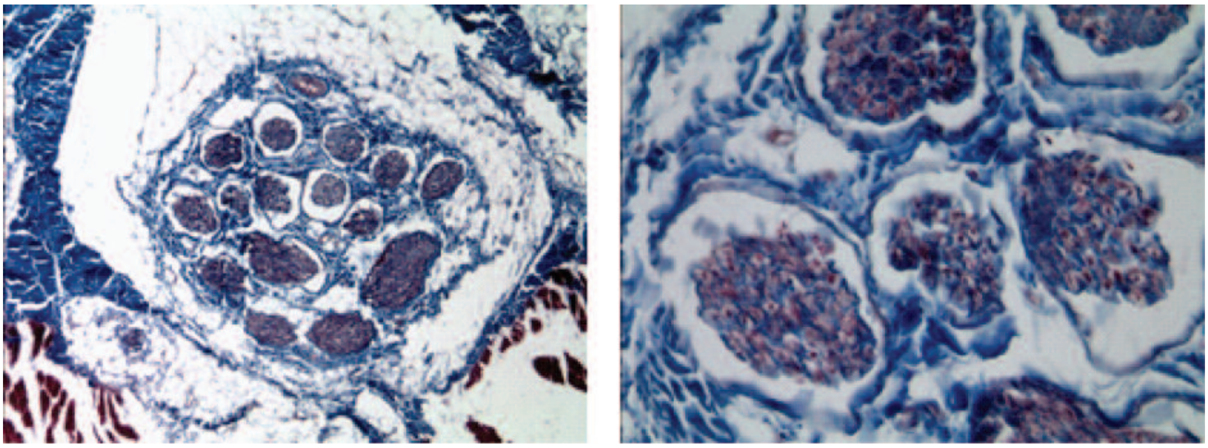
Discussion

Changes in surface temperature and bloodflow of amputated limbs have been associated with the presence of neuropathic pain, both in humans and animals (Kristen *et al* 1984; Katz 1992; Eicher *et al* 2006). Eicher *et al* (2006) performed heat stimulation tests between heifers with intact and amputated tails, and reported that tail-docked animals tended to show greater changes in stump temperature than those with an intact tail, in addition to behavioural changes that demonstrate an increase in sensitivity due to the effect of caudectomy in these animals (Eicher *et al* 2006). In the present study, stump temperature of tail-docked lambs (central view) also showed variations in time (Figure 2); at 15 days after tail-docking it was, on average, almost 6°C higher than at 90 days. After 15 days, a progressive decrease in stump temperature was observed up to day 60, which could be due to the decrease in the inflammatory processes associated with the procedure. Between 60 and 90 days, the temperature did not vary significantly, possibly because the inflammatory process was no longer present. These results are related to the degree of inflammation of the stump, whereby 88 and 68% of tail stumps showed medium to high inflammation (15 and 30 days, respectively), while at 60 and 90 days, there were no stumps showing signs of inflammation.

One of the possible explanations for not finding differences in the IT (dorsal view) between undocked and tail-docked lambs at the different sampling points (Figure 1), may be due to inflammatory processes associated with tail-docking being more local and circumscribed to the area of tail-docking. The area being analysed was, perhaps, very extensive and with a greater dispersion of temperature, thereby failing to register the increase in expected temperature for the tail-docked group. Another explanation that could mask the expected increase in temperature in tail-docked lambs, could be the presence of wool in the tail since, in both groups, animals were not sheared prior to or during the study. However, a study in mice by Zhuo (1998) showed that tail amputation did not lead to temperature variations in tail skin for seven days after the procedure in tail-docked mice.

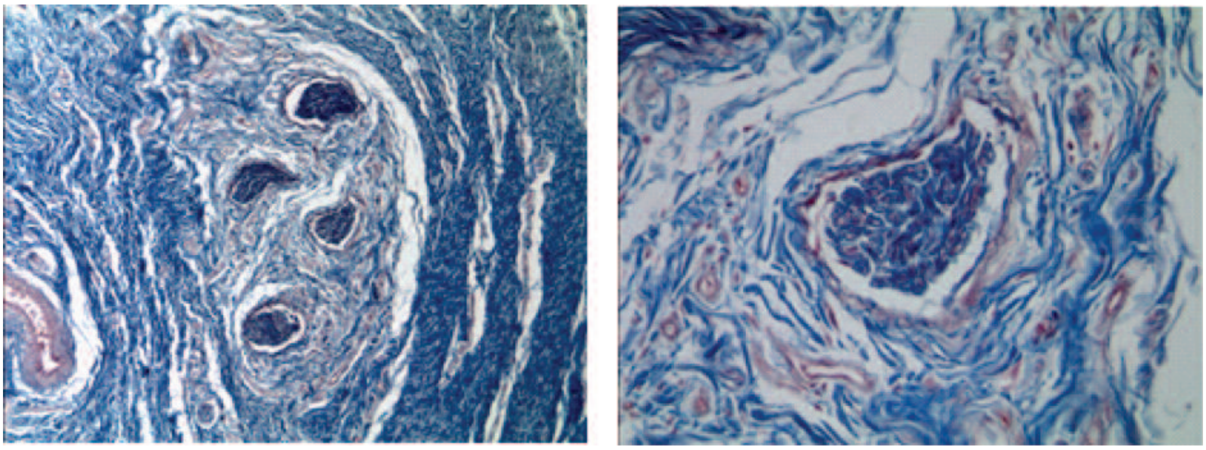
Here, the MNTs of tail-docked lambs showed an increase and correlation with sampling time (Figure 3) — always being lower than that of undocked lambs — suggesting the existence of hyperalgesia associated with tail-docking in the

Figure 6



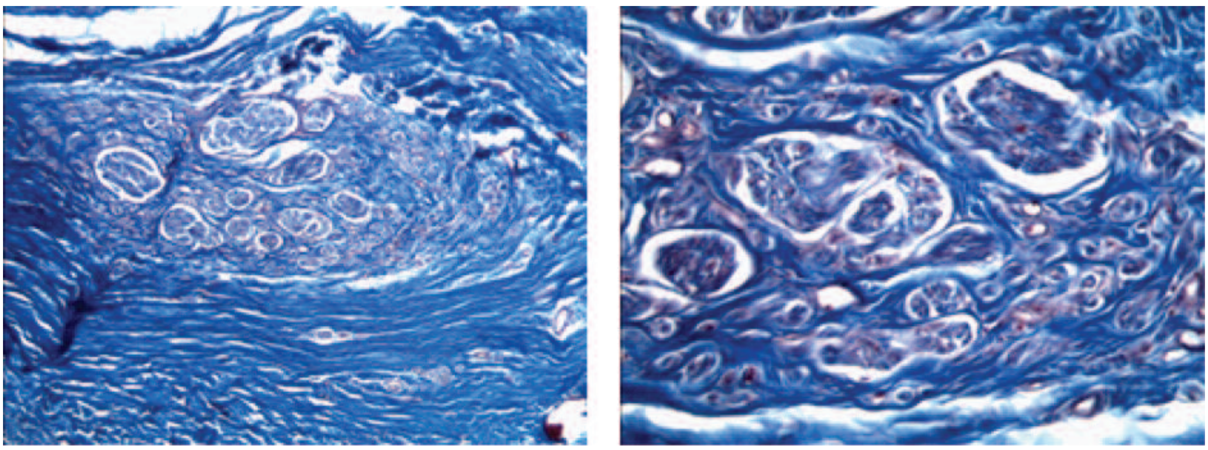
Cross-sectional microphotography of an undocked lamb's tail (control). Score 0 = normal morphology of nerve bundles, without proliferation and fibrosis of epineurial, perineurial and endoneurial tissue with (left) Masson's trichrome, 10× and (right) Masson's trichrome, 40×.

Figure 7



Cross-sectional microphotography of the tail stump of a docked lamb. Score 1 = mild fibrosis of dense fibrous connective tissue in epineurial, perineurial and endoneurial tissue, without disorganised proliferation of nerve bundles with (left) Masson's trichrome, 10×. and (right) Masson's trichrome, 40×.

Figure 8



Cross-sectional microphotography of the tail stump of a docked lamb. Score 3 = marked increase of disorganised nerve bundles of different sizes, surrounded by fibrosis of dense fibrous connective tissue in epineurial, perineurial and endoneurial tissue with (left) Masson's trichrome, 10× and (right) Masson's trichrome, 40×.

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short (15 days) and long term (60 and 90 days). Similarly, Zhuo's (1998) mice study established the existence of mechanical hyperalgesia for at least seven days, whilst the existence of mechanical hyperalgesia up to 16 weeks after tail-docking was reported in pigs by Di Giminiani *et al* (2017); results similar to those obtained here. Perhaps if sampling had been carried out for more than 12 weeks, we may have expected to find hyperalgesia still present; but this was not possible as the animals were sent to slaughter.

This is the first study to establish the presence of hyperalgesia associated with tail-docking in lambs, both in the short and long term (Figure 3). Previously, only the study of Lomax *et al* (2010) had reported primary hyperalgesia in the short term (up to 4 h) but when the procedure was performed with a knife. In contrast, when tail-docking was carried out with a hot iron and a painful stimulus of 75 N was subsequently applied around the wound, short-term secondary hyperalgesia was achieved within 1 min (Lomax *et al* 2010).

Several studies (Lester *et al* 1996; Kent *et al* 2001; Grant 2004) indicate that the hot iron would be a preferable method of tail-docking for lambs with less of a negative impact at the behavioural, physiological and animal welfare levels. In addition, it has been suggested that when cutting and cauterising simultaneously, destruction of nervous tissue and nociceptors is seen in the tail, causing a subsequent loss of sensitivity in the area (Lester *et al* 1996; Grant 2004; Lomax *et al* 2010) and localised pain associated with the procedure (Lester *et al* 1991). However, the results obtained here and those of Di Giminiani *et al* (2017) in pigs, are contrary to those found in the literature which state that animals tail-docked with a hot iron do not experience mechanical hyperalgesia and sensitivity of the stump in the short and long term.

The results of Lomax *et al* (2010) differ with those here, possibly because of differences in instrumentation, anatomical location and sampling times used for evaluation of hyperalgesia. The portable algometer is widely used in the evaluation of neuropathic pain and hyperalgesia (Treede *et al* 2002; Finocchietti *et al* 2011; Duan *et al* 2014) and has a larger surface contact area than the Von-Frey monofilaments used by Lomax *et al* (2010), therefore it has an effect on tissues, intraepidermal nerve endings and deeper nociceptors, establishing tissue sensitisation and the presence of neuropathic pain (Treede *et al* 2002; Finocchietti *et al* 2011). If the activation of deep nervous afferents is required, an instrument that exerts pressure across a wide area should be chosen (Treede *et al* 2002).

The differences in MNTs between groups (undocked/tail-docked) at day 15 can be explained by an ongoing inflammatory process, the sensitisation of the area, and the time elapsed after tail-docking (Figure 3). However, it is suggested that the sensitisation of the area and hyperalgesia do not end with the inflammatory process, because the stump registers a high temperature at days 15 and 30, with low MNTs and is also accompanied by visual signs of inflammation in both sampling times. This is because although at days 60 and 90 there are no visual signs of inflammation (Figure 4) and the stump IT remains low in contrast to days 15 and 30, the tail-docked animals maintain an MNT that is lower than undocked lambs. In addition, between days 60 and 90, there are no significant differences in MNTs of tail-docked lambs (Figure 3), therefore the mechanical hyperalgesia found in this study is a phenomenon that occurs regardless of the time, results that are consistent with those reported by Zhuo (1998) in mice. One possible limitation of this study is the fact that there was only one observer registering behavioural responses of lambs to MNTs, hence there is no possibility of calculating intra- or inter-observer reliability. Therefore, in future studies it might be useful to either use at least two observers registering behavioural responses or video recordings in order to repeat behavioural assessments.

In human medicine, a diagnostic criterion in terms of time to classify post-operative, chronic pain is at least two months after the surgical procedure (Joshi & Ogunnaike 2005). In animals destined for production, Molony and Kent (1997) define chronic pain as that which persists beyond the expected time of healing of a lesion. The present investigation proved the existence of increased sensitivity up to three months after tail-docking of lambs and showed that after three months none of the animals showed signs of inflammation. Therefore, the presence of hyperalgesia at three months can be used as an indirect indicator of chronic pain associated with tail-docking. It is possible that hyperalgesia is a process that occurs independently of the age at which painful practices are carried out (Di Giminiani *et al* 2017). However, it has been demonstrated that there is an increased inflammatory reaction when animals are subjected to painful husbandry practices at older ages (Kent *et al* 1990). Therefore, European and Chilean legislation mandate performing these practices in young animals (Larrondo *et al* 2018). The common age at which tail-docking is carried out in Chile is 3.4 (± 1.9) months (Larrondo *et al* 2018). The negative correlation found between the MNTs and the IT of the tail stump of tail-docked lambs (Figure 5), indicates that the higher the temperature of the stump, the lower the pressure at which the animals respond. This is perhaps associated with the inflammation of the area and a greater sensitisation, since the lowest values of MNTs and the highest values of the stump were recorded at days 15 and 30 (Figure 3). However, after 30 days, the temperature of the stump decreased and the MNTs of tail-docked lambs were still significantly lower than those of undocked animals, suggesting that the mechanical hyperalgesia experienced by docked lambs is a process that occurs independently of the temperature. These results are in accordance with the findings of Zhuo (1998).

The degree of inflammation in the stump of tail-docked lambs significantly influenced the MNTs at 15 and 30 days post-tail-docking. Regardless of the sampling time (15 or 30 days), those animals with an absence of inflammation (grade 0), showed MNTs significantly higher than lambs with intense inflammation (grade 3).

Perhaps, inflammation levels 1 and 2 are somewhat similar and could be grouped into a single category.

According to the designated scale of inflammation for tail stumps, almost all tail-docked animals presented inflammation (grade 1 to 3) at day 15, a finding that was expected due to the amount of time elapsed after tail-docking. However, at day 30, almost half (44%) of the tail stumps showed an inflammation classified as intense and only 20% showed healing. Considering the low MNTs at this sampling time and up to 90 days, it is suggested that the procedure induces inflammatory changes and pain in the lambs. In sheep breeding systems, it is unlikely that veterinarians would check animals after the procedure and use analgesic and/or anti-inflammatory therapy (Viñuela-Fernández *et al* 2007; Dwyer 2008), due to the difficulty in identifying and evaluating pain in this species (Manteca *et al* 2017), not to mention economic and practical reasons (Viñuela-Fernández *et al* 2007). Moreover, since lambs are slaughtered at an early age it might be suggested they experience pain for almost their entire lives, a clear indication of a poor welfare condition (Fitzpatrick *et al* 2006; Llonch *et al* 2015).

Histopathological findings of the present study were similar to those reported in peripheral nerve transection studies, in different animal species (French & Morgan 1992; Eicher *et al* 2006; Sandercock *et al* 2016). The mild nerve bundle proliferation and fibrosis (neuromatous tissue development) observed in 60% of the tail-docked samples, could be associated with an incomplete neuroma formation in lambs at this age, similar to what was reported by Sandercock *et al* (2016) in pigs of four months of age after tail-docking. However, the marked peripheral neural proliferation and fibrosis observed in 20% of tail-docked samples, confirms the presence of neuroma formation in these tail stumps. These results are similar to those reported by French and Morgan (1992) in four to six month old lambs, with an equal traumatic neuroma incidence (20%; 2/10).

Previous studies in lambs (French & Morgan 1992; Fisher & Gregory 2007), have reported the presence of traumatic neuroma in 2/10 (French & Morgan 1992) and 15/26 (Fisher & Gregory 2007) tail stumps. The above-mentioned studies suggested the presence of traumatic neuroma could have implications for animal welfare, however they did not confirm it. This is the first study in lambs that has managed to establish the presence of traumatic neuroma formation as possible sources of long-term hyperalgesia and neuropathic pain after tail-docking. Both of which have negative implications for animal welfare.

Animal welfare implications

The presence of different degrees of inflammation and formation of traumatic neuroma in the tail stumps of tail-docked lambs can be related to acute and chronic pain in these animals. Considering the improbability of veterinarians applying analgesia and/or anaesthesia during and/or after this procedure in extensive ovine breeding systems, the short- and long-term welfare of lambs would appear to be negatively affected.

Conclusion

It is concluded that tail-docking induces acute and chronic pain in lambs for at least 90 days, according to algometry. The hyperalgesia experienced by tail-docked lambs is a chronic process and might occur even when the tail stump is fully healed. In addition, MNTs and the degree of inflammation of the stump is related to IT of the stump at 15 and 30 days after tail-docking. Faster behavioural responses to the mechanical pressure tests and hence greater pain are observed when the degree of inflammation of tail stumps and IT increase.

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