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Castration pain and pain alleviation in semi-domesticated reindeer

(Rangifer tarandus tarandus)

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ACADEMIC DISSERTATION

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To my beloved ones

*"Erämaa, erämaa,
siellä paimen on kuin kotonaan.
Vaikeaa asustaa
siellä oudon on, ja kulkea.
Aniharva kulkijoista sinne yksin uskaltaa,
Ei siellä rikkautta saa, mutta rauhan tavoittaa.
Maa hurjien veikkojen,
korppi soittaa kuolinkellot heikkojen."*

*"Wilderness, wilderness,
there the herder feels at home.
Ruthless for a stranger
to live and roam.
Very few of the wanderers dare go alone,
there's no wealth for you but peace is known.
The land of wild brethren,
the raven tolls the death knells for the weak."*

Abstract

Approximately 2500–4000 reindeer (*Rangifer tarandus tarandus*) bulls are castrated annually in Finland, mostly without pain alleviation, in round-ups or winter enclosures. Pain is an important welfare issue and research on other ruminants shows that clamp castration is painful. Reindeer, as prey animals, show modest castration pain. Recognizing pain, understanding the importance of pain alleviation and having appropriate methods and motivation for providing analgesia are all critically important. According to new animal welfare legislation (693/2023), pain alleviation in painful procedures is mandatory in Finland from January 2024 onward. There has been a gap in knowledge on reindeer castration pain behaviours and on the practical means of medicating reindeer, particularly that targeting castration pain alleviation. These semi-domesticated, free-ranging animals are not very used to human presence, they are easily excitable, field anesthesia is problematic and capture and prolonged restraint may cause problems, even mortality. Reindeer are prone to stress induced hyperthermia, gastric ulcers and capture myopathy.

Pain alleviation in reindeer is challenging. Their wintertime metabolism can slow down by more than 50%, which may have a substantial impact on the pharmacokinetics of different drugs. Sedation and/or narcosis can cause serious side-effects in wild ruminants, especially during harsh winter conditions and hectic round-up situations. Many of the sedative drugs available, especially sedative antagonists, are not accepted for production animal use. Local anesthesia (LA) adds to handling time or handling occasions. The evidence-base is missing on the use of non-steroidal anti-inflammatory drugs (NSAID) in reindeer. These drugs are now used off-label according to the cascade rules of directive EU (2019/6), extrapolating the doses from those for other ruminants.

The work embodied in this thesis aims to define pain behaviours and investigate best present pain alleviation practices in reindeer castration. More specifically, the aim is 1) to investigate the wintertime pharmacokinetics of NSAID meloxicam on reindeer after intravenous (IV) and oral dosing, and 2) to investigate the effects of castration on reindeer behaviour, rectal temperature and respiratory rate during the clamp castration procedure, and determine how these are affected by handling, LA and NSAID, and 3) to investigate the effects of clamp castration on activity and daily rhythm of reindeer during the initial hours and up to three days post procedure, and how these are affected by an NSAID.

The thesis presents the first ever study on the pharmacokinetics of an NSAID in reindeer: the plasma meloxicam concentrations measured after both IV and oral administration were within the therapeutic concentration of meloxicam as determined for other species, providing analgesia for up to 2-3 days (I). An ethogram, a description of pain behaviours during clamp castration in reindeer, was created. Reindeer express castration pain by contracting their abdominal muscles and closing their nostrils (i.e. holding breath), and showing locomotory patterns that might indicate pain-induced change in their activity rhythm in the first days following the procedure. LA with a 5-minute effect time failed to

reduce the pain expression behaviours, while the longer handling time waiting for the LA effect caused problems with capture and restraint stress, that is, signs of intimidating hyperthermia (II). NSAID meloxicam does not ease the immediate pain during clamp castration, but it is beneficial for castrated reindeer, changing postoperative activity patterns in a way that suggests pain alleviation during the first 2-3 days following clamp castration (III).

Further research is needed, with more sensitive measures of pain related to castration, and the effective means of pain alleviation, or scientific innovations or practices of avoiding painful procedures completely. Future experiments are also needed on the efficacy of meloxicam, evaluating the appropriate dosage and adequacy of analgesia after various dosing routes and to explore protein-binding, tolerance and toxicity.

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Research on bovine calves and lambs confirms that the castration technique of choice is using Burdizzo clamps, due to a faster healing process (Molony et al., 1995; Stafford and Mellor, 2005; Mellema et al., 2006; Melches et al., 2007; Thüer et al., 2007). Compared with the different castration methods (surgical, rubber band), clamp castration causes the least acute pain and distress, especially when alleviated with medication, but wound healing can be extended and the castration itself occasionally fails (Stafford et al., 2002). It has been shown that clamp castrated calves may suffer for the first few hours (King et al., 1991; Robertson et al., 1994), but after clamp castration, long-term chronic pain occurs less frequently. Molony et al. (1995) showed that after Burdizzo castration, swelling increased slightly for two days, and then decreased, disappearing within 15 days post procedure. According to Thüer et al. (2007), local swelling after Burdizzo castration in calves varied from moderate to severe, reaching a maximum during the first two days, lasting an average of 15 ± 7.7 days ($\pm$ SD). Several studies on veterinarian attitudes towards clamp castration pain conclude that clamps are assumed to cause 6-9 strength of pain on scale 1-10, where 1 indicates no pain and 10 the worst possible pain (Tschoner, 2021). It is noteworthy that reindeer are, unlike other ruminants, castrated as adults. Burdizzo clamp castration can cause an increasing stress response in older animals (King et al., 1991; Ting et al., 2003), and tissue damage is more distinct (Pieler et al., 2013).

Currently there are no alternative methods for castrating reindeer. Rubber band castration is considered even more painful with weeks-long (minimum 42 days) afterpain (Molony et al., 1995; Melches et al., 2007). The rubber ring prevents blood flow and results in atrophy and drop off the testes and distal scrotal tissue after 4-6 weeks (Mellor and Stafford, 2000). A rubber band should not be used for animals older than one week of age; the risk of complications, pain and distress are considerably higher in older animals (Molony et al., 1993). Fortunately, rubber band castration is not accepted in the Finnish national animal welfare legislation. Surgical castration with sedation and/or anesthesia is a veterinarian act only permitted by legislation, and secondly, it negatively affects antler growth: the antlers are small, deformed and remain covered with skin (Nikolaevskii, 1961, Marliani et al., 2023). Moreover, lack of circulating endogenous testosterone in adult bulls can cause the persistence of velvet antlers growing disorganized, creating a “perruque”, i.e. a wig antler, which negatively impacts welfare, creating favourable conditions for myiasis (infection of a fly larva) and secondary infections (Fletcher et al., 2016).

List of original publications

This thesis is based on the following publications:

- I** Nurmi H, Laaksonen S, Raekallio M, Hänninen L, 2022. Wintertime pharmacokinetics of intravenously and orally administered meloxicam in semidomesticated reindeer (*Rangifer tarandus tarandus*). *Vet Anaesth Analg*. 2022 Jul;49(4):423-428. doi: 10.1016/j.vaa.2022.04.005. Epub 2022 Apr 29. PMID: 35659721
- II** Nurmi H, Laaksonen S, Häättilä T, Valros A, Sauvala M, Hänninen L, 2022. The impact of clamp castration on the behaviour and body temperature of reindeer (*Rangifer tarandus tarandus*) – effects of local anesthesia and non-steroidal anti-inflammatory drug. *Applied Animal Behaviour Science*, Volume 255, 2022,105719, ISSN 0168-1591
<https://doi.org/10.1016/j.applanim.2022.105719>.
- III** Nurmi H, Hänninen L, Laaksonen S, Valros A, (2024). The effect of non-steroidal anti-inflammatory drug on the distance travelled by reindeer (*Rangifer tarandus tarandus*) in the wilderness after clamp castration – a pilot study. Submitted manuscript for *Acta Vet Scand* May 1, 2024.

The publications are referred to in the text by their roman numerals. The original articles are reprinted with the permission of the publisher Elsevier.

1 Introduction

Reindeer husbandry is a centuries old practice that remains vital to livelihoods in northern Finland, with 190 000 reindeer grazing freely over landscapes covering 36% of Finland's total area. This is referred to as the Reindeer Husbandry Area. Regulation of this traditional livelihood, essentially a pastoral system, is based on the Finnish Reindeer Husbandry Act (848/1990). The geographical area used for reindeer herding in Finland is divided into 54 reindeer herding cooperatives that maintain several unique traditions (Figure 1).

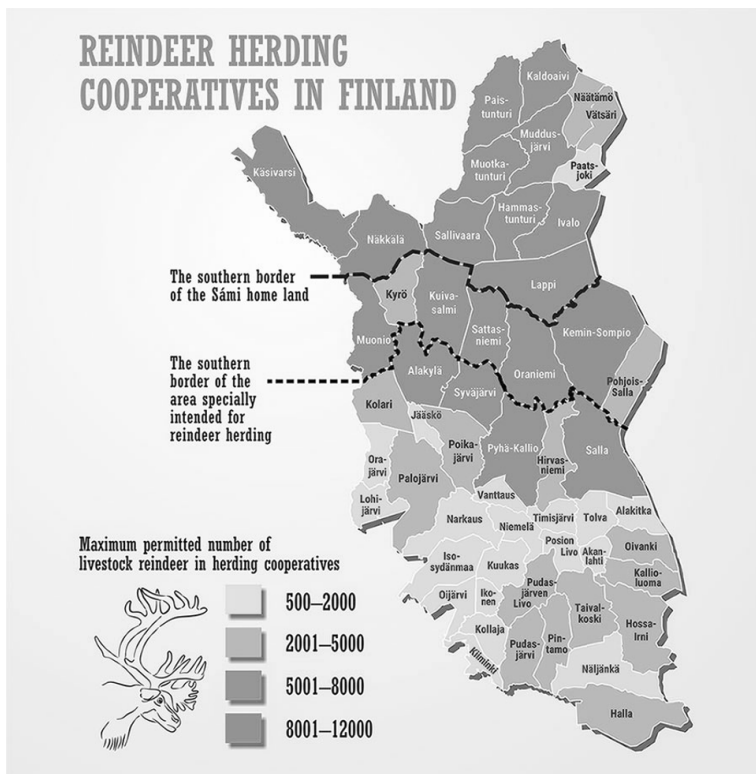


Figure 1 Reindeer herding area and cooperatives in Finland. From the Reindeer Herders' Association, 2024.

Reindeer herding practices in Fennoscandia are highly diverse, varying from the long-distance migrating tundra type herding in Norway and Sweden to shorter distance migration and more stationary taiga type herding in most of Finland (Riseth et al., 2019). In Finland, reindeer herding practices follow the rhythm of the northern nature, and the natural behaviour of reindeer is considered in the herding work. The freely grazing herds are transferred from the wild to round-up enclosures twice a year, in the summer for calf marking and, in the fall and winter for the round-ups, where the reindeer are counted, earmarked, treated with antiparasitic drugs and separated for slaughter. A share of the reindeer is kept in separate winter enclosures or unfenced areas for supplemental feeding during the coldest winter months, especially in the southern parts of the reindeer husbandry area. However, most of the animals are herded grazing freely in the wild all year round (Holand et al., 2022; Reindeer Herders' Association, 2024) and are therefore less accustomed to human presence.

According to estimates from the Reindeer Herders' Association statistics, approximately 2500–4000 reindeer bulls are castrated annually in Finland, mostly without pain alleviation, in round-ups or winter enclosures. Castration is performed by crushing the spermatic cord with emasculator clamps for 6–7 seconds per side (Laaksonen, 2019) and is justified by better slaughter condition (Wiklund, 2008) when these semi-domesticated animals are gathered from the grazing lands in the years following castration. The rut – the period in the fall during which the reindeer bulls are sexually active and fight for the females – is wearing for the intact males, but also assists the herding work because reindeer bulls create harems, gathering the animals into larger herds that are easier to lead into round-up enclosures. Based on primarily anecdotal evidence, castrated reindeer are more manageable and tend to survive better during the rutting season, without excessive weight loss and fighting injuries. Moreover, intact males cannot be slaughtered during the rut for several reasons: handling and transporting of the aggressive bulls is demanding, and hormonal changes and mating behaviour (e.g. urinating on the hind limbs) create malodour and off-flavour of the meat (Rowell and Blake, 2019). The male reindeer are mostly castrated at around five years of age, when they are no longer needed for breeding purposes. Similarly, some younger bulls are taken out of breeding and castrated. Tourist draught reindeer are mostly castrated at the age of three to four years as a part of their taming process and because of their impressive velvet antlers that are attractive to tourists – intact males drop their antlers soon after the rut every year, castrates later in spring (Fletcher et al., 2016; Laaksonen, 2019; Soppela et al., 2022).

Research on other ruminants shows that clamp castration is clearly painful (Robertson et al., 1994; Fisher et al., 1996; Bretschneider, 2005; Stafford and Mellor, 2005). Animals struggle, their heartbeat and respiratory frequencies rise, they move less or stand more after castration, or the movement and standing

positions show typical pain-related stiff standing postures (Molony et al., 1995; White et al., 2008; Currah et al., 2009; Brown et al., 2015). Moreover, untreated pain can cause restlessness and increases in locomotor activity (Molony and Kent, 1997), and alterations in activity patterns like resting and sleeping behaviours (Ruckebusch, 1975; Siegel, 2005; Berger, 2011). No studies exist on how acute castration pain alters reindeer behaviour.

Pain is a significant welfare issue: recognizing pain, understanding the importance of pain alleviation and having appropriate methods and motivation to relieve pain are all critically important (Weary et al., 2006). Measuring a subjective pain experience is not easy, and pain is easily unrecognized, especially with stoic prey species like reindeer, whose evolutionary survival strategy is based on not showing weakness to predators (Currah et al., 2009). Nevertheless, difficulties in recognizing and evaluating concealed pain behaviours do not undermine the reasonable assumption that all mammals undergo painful procedures similarly, sharing comparable neural pathways for pain sensation (Weary et al., 2006; Hudson et al., 2008; Tschoner, 2021). It is possible to approach pain scientifically and define how the animal's physiology and behaviour alter during and following painful stimuli (Weary et al., 2006; Stewart et al., 2010).

In practice, castration pain can be managed with local anesthesia (LA), with or without sedation and/or narcosis, and postoperative pain treated with peroral or injected nonsteroidal anti-inflammatories (NSAIDs) (Coetzee, 2011). However, managing castration pain in reindeer is particularly challenging. Reindeer are often released back into the wild immediately after they are treated. In the modern era, reindeer are semi-domesticated production animals that graze freely in large herds and are relatively unused to human presence. Therefore, repeated or prolonged handling must be regarded as unfavourable for reindeer (Rehbinder et al., 1982; Trondrud et al., 2022). Reindeer are easily excitable and different measures connected with herding and handling may cause both physiological and emotional distress (Lian et al., 2019; Trondrud et al., 2022). Captured and restrained reindeer are prone to a stress-induced increase in body temperature, seen as panting, when the brain temperature reaches 39.0 °C (Blix et al., 2011). Moreover, reindeer are susceptible to gastric ulcers (Rehbinder et al., 1982) and capture myopathy, a non-infectious, metabolic disease of wild and domestic animals; a mortal outcome of stress (Paterson, 2014; Breed et al., 2019). To date, castration pain alleviation has not been studied in reindeer.

It is notable that physical restraint in free-ranging wildlife causes greater stress compared with chemical restraint (Boesch et al., 2011; Arnemo et al., 2014), although this is not entirely the case for semi-domesticated reindeer. Unfortunately, field anesthesia of semi-domesticated reindeer is considered problematic: immobilized and anesthetized wildlife species frequently demonstrate serious side-effects, including hypothermia, hyperthermia, hypoxemia, and capture

myopathy (Arnemo et al., 2014; Caulkett and Arnemo, 2015). Most of the drugs used for sedation and anesthesia of wild species, including their antagonists, are not approved for use with food-producing animals (Kreeger and Arnemo, 2012). Monitoring sedated reindeer bulls, which are prone to fighting during the rutting season, would be nearly impossible to arrange within enclosures during autumn round-ups.

Local anesthesia has its challenges as there are at least a few minutes of delay time between the administration and the onset of the analgesic activity (Coetzee, 2011), adding to capture occasions or restraint time and making the use difficult, especially in hectic reindeer round-up conditions. NSAID alone, on the other hand, is not sufficiently effective for reducing the acute pain associated with castration of bovine calves (Stafford and Mellor, 2005; Coetzee, 2011). Therefore, the role of NSAIDs is merely important in treating postoperative pain. No studies have been published concerning the pharmacokinetics of NSAIDs in reindeer.

The animal welfare legislation in Finland has recently been revised and pain alleviation for painful procedures is mandatory from 2024 onwards (Animal Welfare Legislation (693/2023)). The new demands challenge the old herding tradition, and both herder motivation and suitable and evidence-based best practices for pain alleviation are needed to be able to establish the new regulations.

To summarize, research knowledge on how reindeer express castration pain does not yet exist. There is also a gap in research knowledge on the optimal means of medicating reindeer, targeting castration pain alleviation, as these animals are not used to human presence, they are easily excitable, field anesthesia is problematic and capture and prolonged restraint may cause serious problems, even mortality. Furthermore, research knowledge on the pharmacokinetics of analgetic drugs for reindeer does not exist, and doses are extrapolated from those for other ruminants, even though there is considerable variance among different ruminant species.

2 Review of the literature

2.1 Cultural and practical aspects of reindeer herding

Reindeer husbandry is a traditional, centuries old livelihood, a part of the broader cultural and historical context of the Indigenous People of the area, and their traditional way of life that revolves around reindeer herding. Reindeer herders have a deep connection to the land and its resources. The cultural value of reindeer herding is enormous, and reindeer are utilized comprehensively (Holand et al., 2022). This requires that bulls be slaughtered and utilized after they are no longer required for breeding.

Free-ranging reindeer are gathered from natural pastures twice a year – in summer for calf marking to indicate ownership and in fall/winter for slaughter and breeding separation. During summer, the presence of flying blood-sucking insects aids herders as reindeer gather in larger groups to minimize insect pressure (Reindeer Herders' Association, 2024). According to traditional knowledge, reindeer herders avoid slaughtering in summer due to hygiene concerns related to loose hair and bitter perspiration taste, although no such off-flavour has been reported in studies concerning the effect of feed on flavour variation (Wiklund et al., 2019). In the fall, during the rut, bulls form harems, leading to larger, more manageable herds that are easier to gather from the pasturelands to round-up enclosures (Reindeer Herders' Association, 2024). During the rut, slaughtering the males removed from breeding is impractical due to strong odours, poor condition, and the risk of fighting injuries. Traditional bull slaughter takes place immediately before the rutting season (Malmfors and Wiklund, 1996; Skarin et al., 2022).

The Reindeer Husbandry Act (848/1990) regulates reindeer herding in Finland: the animals must be released for free ranging no later than 15 June if they have been kept in separate winter enclosures for supplementary feeding and/or calving. Based on this legislation, the Ministry of Agriculture and Forestry limits the number of breeding reindeer in cooperatives, and a herder is considered full-time when owning a minimum of 80 breeding reindeer, which is the threshold for receiving state support in Finland. State support to reindeer herders is considerably lower than that for general agriculture. The role of compensation (predation and traffic accident losses, pasture degradation) has grown in recent decades (Holand et al., 2022).

2.2 Welfare and significance of accumulating stress in reindeer

The definition of animal welfare is broad and has been disputed over recent decades, but usually it centers around the animal's ability to cope with the ambient conditions and events in its surroundings, based on individual emotions. Feelings, such as pain, fear and pleasure play a major role in welfare and in various coping strategies targeted to controlling mental and bodily stability. For scientists and legislators, welfare is a potentially measurable quality of living at a particular time (Broom, 2014), and this approach is also used in this thesis.

Understanding the significance of stress is key to securing the welfare of reindeer and controlling diseases and promoting health. Reindeer are highly susceptible to stress, and stress induced problems (Rehbinder, 1990). Numerous environmental changes in the physical landscape, such as mining, forestry, tourism, housing, industry, and agriculture, contribute to stress in reindeer. Additionally, various factors, including threats, interference, fear, excitement, chasing, human presence, large carnivores, and hunting or herding dogs, can induce stress in this semi-domesticated species. Stress can also arise from different herding and handling practices, including gathering animals with helicopters, snowmobiles and all-terrain vehicles, as well as treatments causing pain and restraint, transport, corral confinement, ambient conditions, improper feeding, lack of water, exceptional climate conditions, insect infestations, and separating calves from their mothers (Lian et al., 2019). Stress tends to accumulate. Reindeer in general are a rather vigilant and flight-reactive species (Baskin and Hjalten, 2001; Reimers et al., 2011; Nieminen, 2013). It is, however, difficult to estimate what animals experience based on flight reaction only as even reindeer not actively attempting to escape may experience severe emotional distress (Rehbinder et al., 1982; Rehbinder, 1990).

Handling and restraint stress has been studied in various species, and habituation may ease stress symptoms (Chen et al., 2015). Domestication is a continuous, long-term genetic process (Larson and Burger, 2013; Jensen and Wright, 2013) and traditional old-time reindeer herding with small herds and daily contact with the animals contributed to comparatively domesticated animals (Rehbinder, 1990; Røed et al., 2021). However, modern-times free-grazing reindeer are not as used to human presence and manual handling, and restraint can be a major stress factor for less tame reindeer, causing stress lesions like abomasal hemorrhages, and muscular and myocardial degeneration (Rehbinder et al., 1982; Rehbinder, 1990, Wiklund et al., 1996a,b).

Hence, reindeer are susceptible to capture myopathy (CM), a non-infectious metabolic disease that affects both wild and domesticated animals, resulting in morbidity and mortality due to stress induced by factors such as fear, pain, pursuit, capture, restraint, and transportation (Paterson, 2014; Breed et al., 2019). Capture-

related elements that contribute to CM include high chase speed, prolonged exertion without rest, excessive handling, prolonged restraint, restraint that promotes struggling from unnatural positioning, stress, pain, fear, and voices (Paterson, 2014). The diagnosis is only possible post-mortem, but it is suspected to be one of the causes of losses following treatments.

Castration and castration pain represent one of the apparent, but not yet studied, stressors in reindeer.

2.3 Castration in reindeer herding practices

2.3.1 Castration in general

Traditionally – anecdotally until the 1960s-1980s – reindeer were castrated by biting and chewing the testicles. In modern-times, reindeer are routinely castrated by crushing the spermatic cord with emasculator (Burdizzo) clamps. “Biting” is still a dialect term for clamp castration (Soppela et al., 2022).

Castration is justified by better slaughter carcass condition (Wiklund, 2008) when the year-round freely grazing animals are gathered from the grazing lands in the years following castration. According to mostly anecdotal information, castrated animals are easier to handle and survive the rutting period better and they are therefore found from the grazing lands in a better condition. Castrated animals are calm, and have therefore a function in herd control, and the castrates are also the strongest, surviving the harsh winter conditions with their ability to dig and break ice (ICR, 2024). Castration may have some influence on the meat quality, especially on the fat layer and the fatty acid content of muscle, as shown in wapiti (*Cervus canadensis*) (Sang-Woo et al., 2016).

The male reindeer are mostly castrated in Finland at around five years of age, when they are no longer needed for breeding. Five years is also the optimal slaughter age for male reindeer from ecological and economical perspectives (Pekkarinen, 2018). Some genetically or physically inferior younger bulls are taken out of breeding and castrated. Tourist draught reindeer are mostly castrated at the age of three to four years, and racing reindeer a little later, if at all, as intact reindeer are more active than castrates (Laaksonen, 2019, Soppela et al., 2022). Approximately 1/3 of the castrated animals are sleigh-pulling tourist reindeer (estimated from the Reindeer Herder’s Association annual statistics 2022-23).

Castration in Finland has been performed mostly without pain alleviation (Animal Welfare legislation 247/1996), except for certain tourist reindeer, whose owners have followed the ethical rules of the tourism industry (Green Key, 2023) and voluntarily used analgesia in castration. According to the new animal welfare legislation in Finland, pain alleviation for painful procedures is mandatory from January 1st, 2024 (Animal Welfare Legislation (693/2023)). Clamp castration is still

accepted by law in the reindeer husbandry area, when performed by a skilled herder using appropriate equipment and mandatory NSAID. Outside of the reindeer husbandry area, castration must be performed by a veterinarian.

The legislative demands regarding castration vary among the Nordic countries. According to the Norwegian Animal Welfare Act (2009), use of anesthesia is mandatory in reindeer castration, even when castrated in the field using the bloodless Burdizzo method, making it a veterinarian act only. This protocol has been protested by the Sámi society (Magga et al., 2009). Sweden allows reindeer castration with Burdizzo clamps, without local anesthesia and without the need to consult a veterinarian or other animal health personnel (Djurskyddsförordning 2019:66).

2.3.2 Castration methods

In Finland, the only legislated castration method for reindeer is using the closed technique with emasculator (Burdizzo-type) clamps (Figure 2). With the newest emasculator clamp models, the procedure time is short, less than 30 seconds (Jordbruksverket, 2009). Reindeer are usually pushed over on to their side with the upper hind limb pulled forward for better visibility, and the *vasa deferentia* is palpated, with care taken not to break the skin. Clamps are used to crush the spermatic cord for 6–7 seconds per side, after which the testicles gradually wither away (Laaksonen, 2019). In bovine species, atrophy of the testes occurs in 4-6 weeks after preventing the blood flow to the testes (Mellor and Stafford, 2000; Stafford et al., 2002). One single crush on the scrotal neck is not recommended due to incomplete castration success (Boesch et al., 2008), but instead one crush per spermatic cord is preferable with respect to animal welfare according to Thüer et al. (2007). The latter technique saves part of the blood circulation, preventing necrosis of the scrotum (Figures 2 and 3).

Currently there are no alternative methods for castrating reindeer. Rubber band castration is considered even more painful with weeks-long (minimum 42 days) afterpain (Molony et al., 1995; Melches et al., 2007). The rubber ring prevents blood flow and results in atrophy and drop off the testes and distal scrotal tissue after 4-6 weeks (Mellor and Stafford, 2000). A rubber band should not be used for animals older than one week of age; the risk of complications, pain and distress are considerably higher in older animals (Molony et al., 1993). Fortunately, rubber band castration is not accepted in the Finnish national animal welfare legislation. Surgical castration with sedation and/or anesthesia is a veterinarian act only permitted by legislation, and secondly, it negatively affects antler growth: the antlers are small, deformed and remain covered with skin (Nikolaevskii, 1961, Marliani et al., 2023). Moreover, lack of circulating endogenous testosterone in adult bulls can cause the persistence of velvet antlers growing disorganized,

creating a “perruque”, i.e. a wig antler, which negatively impacts welfare, creating favourable conditions for myiasis (infection of a fly larva) and secondary infections (Fletcher et al., 2016).



Figure 2 The only legislated reindeer castration method in Finland is using the closed technique with emasculator (Burdizzo-type) clamps. Photo: Kaija Länsman/YLE.

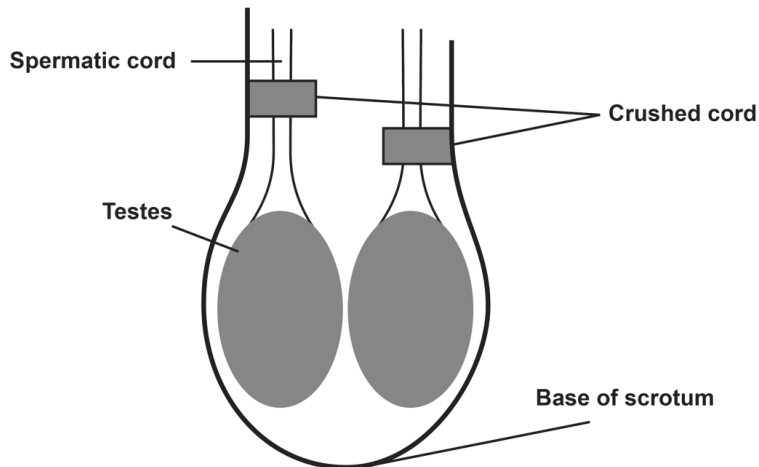


Figure 3 Castration is performed by crushing the spermatic cord and skin from both sides separately, at a different level.

2.3.3 Alternatives to castration

Pain could be prevented also by evaluating the necessity for a painful procedure (Weary et al., 2006). Avoiding castration is not always possible, however, slaughtering the males intact before or after the rut and rehabilitation is an option in some circumstances and areas. Nevertheless, the former option wears the pasture lands in vain, that is, slaughtering the, in any case few, breeders before the breeding season, and the latter option adds to feeding costs if reindeer are kept in enclosures for recovery following the rut. Therefore, from an economics point of view, this solution is not straightforward. As a part of our reindeer welfare project (PORKAT 2019-2023), we designed a questionnaire about reindeer herder attitudes to castration (not yet published). The issue of slaughtering reindeer bulls intact divided the respondents: out of 216 responses, 52.7% would consider it possible to slaughter the males intact (many already do this after recovering from the rut), and the remainder considered it not possible due to free grazing in enormous pastures, the quality of the meat, lack of workers and stressful additional animal capture. The heterogeneity of opinion over the reindeer herding area was represented in the responses.

Immunocastration, an active immunization against gonadotropin-releasing hormone (GnRH, gonadoliberin), may represent possibilities in the future, however, the current protocol for piglets requires repeated injections at a maximum of 5 months apart (Zamaratskaia et al., 2008; EMEA, 2009), which is not suitable in most areas of reindeer herding due to the nature of the reindeer herding practices with free-ranging animals. Notably, this vaccination is administered to very young pigs, already slaughtered at 6 months of age, compared with reindeer that are castrated as full-grown adults. In the case of older pigs, a three-dose vaccination regimen, or even more doses, might be required (Čandek-Potokar et al., 2017). Animals must be caught for handling and vaccination. Preliminary demonstrations of effective castration of reindeer bulls with anti-GnRH vaccine have been claimed in Norway (Magga et al., 2009). Moreover, Marliani et al. (2023) recently suggested that a deslorelin acetate implant could work as a non-invasive contraception approach in human-managed populations of deer (*Cervus elaphus* and *Dama dama*), although with a small sample size. In pork production, consumer acceptance of immunocastration has also been questioned by stakeholders in the pork chain, preventing its large-scale implementation. However, a survey made in 23 European countries indicated the acceptance of immunocastration to be high (71 %), although the results may be biased due to convenience sampling with professionally involved stakeholders as respondents (Aluwé et al., 2020).

2.4 Castration pain and pain assessment

2.4.1 Understanding pain in ruminants – insights from research on clamp castration and analgesia

As reviewed by Sneddon et al. (2014), there is no reasonable doubt that all mammals experience pain. Nociceptive stimuli (e.g. high mechanical pressure, irritating chemicals, temperature changes) alert the animal to actual or potential tissue damage and lead to physiological and behavioural changes. Physiological response is mediated by the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, causing autonomic changes in heart rate, respiratory frequency, blood pressure, body temperature and weight, and catecholamines (adrenalin, noradrenalin) or glucocorticoids. Behavioural signs of animal pain are more complex.

Pain perception is always subjective, affected by several issues, including age, experience, other individuals, surroundings and pain-causing conditions. Therefore, pain is not easy to measure. It has been beneficial during evolution that many animal species do not clearly express pain, trauma or illness. Several types of response can be recognized: 1) learned avoidance of recurrence of the experience; 2) withdrawal reflexes, 3) responses that minimize pain and assist healing (e.g. lying or standing still); and 4) communication by vocalization, posture, or other means such as smell (Molony and Kent, 1997; Weary et al., 2006). Moreover, untreated pain causes restlessness and increases in locomotor activity (Molony and Kent, 1997), and alterations in resting and sleeping behaviours (Ruckebusch, 1975; Siegel, 2005; Berger, 2011). Hokkanen (2015) suggested that untreated disbudding pain in calves could disrupt adapted activity rhythms. Prey animals are generally very impassive in their pain response because expressing pain does not confer a biological advantage (Currah et al., 2009). Pain responses can be used to assess whether a certain procedure is painful in general, and to test efficacy of a given analgesia. At the individual level, these responses show whether an animal suffers from pain and whether the pain alleviation provided is sufficient (Weary et al., 2006).

Based on research performed on other ruminants, clamp castration, like all the other castration methods, is considered to be painful (Robertson et al., 1994; Fisher et al., 1996; Bretschneider, 2005; Stafford and Mellor, 2005). Bovine calves and lambs express acute pain during castration, with foot stamping, tail wagging (Robertson et al., 1994), struggling, kicking (Thüer et al., 2007; Melches et al., 2007), head and leg movements, urinating or defecating and vocalization (Melendez et al., 2018). Animals struggle and their heart rate and respiration frequency rise. Non-treated pain makes bovine calves less active during the days following treatment – they move less and stand more after castration, or the

movement and standing position indicate pain (Currah et al., 2009; Molony et al., 1995; White et al., 2008; Brown et al., 2015).

A summary of the scientific literature examining the impact of clamp castration and analgesia on behaviour or other parameters in ruminant species is presented in Table 1. The summary is restricted to research where clamps are at least in one group used alone and not combined with a rubber band.

Table 1 Summary of the scientific literature examining the impact of clamp castration and analgesia on behavior or other parameters in ruminant species.

Surgical = Su, Burdizzo = Bu, Control = C, Rubber ring = RR, SR = Partial scrotal resection, LA¹= Lidocaine, LA²= Bupivacaine, LA³= Saline, LA⁴ = Procaine hydrochloride, NSAID¹= Ketoprofen, NSAID² = Carprofen, NSAID³ = Flunixin meglumine, NSAID⁴ = Ramifenazone/Phenylbutazon Xyl = Xylazine

Ref.	Animals	Analgesic protocol	Castration techniques	Prior medication	Outcomes
King et al. 1991	Beef calves 78 ± 12 d or 167 ± 14 d of age	-	Su, Bu, C	-	Cortisol greater post castration in surgical than Burdizzo, not differing from Control.
Robertson et al. 1994	Calves 6, 21, 42 days old	-	Su, Bu, RR, C	-	Cortisol peak for all groups, shortest with Burdizzo. All methods caused acute pain, least with Burdizzo.
Molony et al. 1995	Dairy calves 1 week of age	-	Su, Bu, RR, Bu + RR, C	-	All methods increased cortisol more than C, Su highest, RR longest. Severe long-term swelling and inflammation with RR. Immobile postures for Bu and Su, considerable acute and chronic pain with RR (licking, abnormal standing, head and hind leg movements).
Fisher et al. 1996	Dairy calves 5.5 months	LA ¹	Bu ± LA, Su ± LA, C	15 min	Surgical castration induced a greater and prolonged plasma cortisol response than Burdizzo castration, LA reduced it especially for surgical group. Feed intake decreased for both castration methods.

Ref.	Animals	Analgesic protocol	Castration techniques	Prior medication	Outcomes
Murata 1997	Dairy calves 3–4 months		Bu	-	Blood lymphocyte parameters, as well as plasma cortisol levels, can be affected by Burdizzo castration.
Stafford et al. 2002	Dairy calves 2–4 months	LA ¹ , NSAID ¹ IV+LA ¹	RR, Band, Su, Bu	20 min	Clamp castration caused the smallest cortisol response, reduced or eliminated by LA or LA+ ketoprofen.
Ting et al. 2003	Dairy calves 13 months	NSAID ¹ IV, LA ¹ , Xyl + LA ¹ epidural	Bu	20 min	Burdizzo increased plasma cortisol and acute-phase proteins and suppressed immune function and growth rates. Ketoprofen was more effective in reducing inflammatory responses. Ketoprofen and epidural lidocaine were more effective than LA in decreasing pain-associated behavioural responses during the first 6 h after treatment.
Zulauf et al. 2003	Calves (Simmental + Limousine) 110–160 kg	Xyl+NSAID ⁴ + LA ¹ , castrated ± analgesia, non-castrated ± analgesia	Bu	15 min Xyl and NSAID ⁴ , 5 min LA ¹	A positive effect after administration of a NSAID was obvious for the serum cortisol concentration and scrotal swelling. A positive effect on the concentrate intake within the first 3 days after castration.
Pang et al. 2006	Dairy calves 5.5 months	NSAID ² IV	RR, Bu, C	20 min	Both castration methods increased plasma cortisol and acute-phase protein production moderated by carprofen administered before castration.
Mellema et al. 2006	Lambs 2–7 days old	±LA ¹	Bu, RR, C	5 min	LA reduced significantly the peak cortisol, especially in RR. Bu pain scores high during castration, only

Ref.	Animals	Analgesic protocol	Castration techniques	Prior medication	Outcomes
					partially reduced by LA. LA reduced active behaviours postop.
Melches et al. 2007	Lambs 10-week-old	LA ¹ LA ²	Bu, RR, Su, C	10–15 min	Bu and Su showed significant acute pain response, LA had no effect on response. Healing fastest with Bu, a few days. Bu with LA recommended for lambs over 10 weeks old.
Thüer et al. 2007	Dairy calves < 1 month	LA ¹ LA ³ Saline	Bu, RR, C	5 min	Cortisol levels increased significantly without LA. Abnormal postures and pain-indicating active behaviour were seen significantly more frequently after Burdizzo castration ± LA than in sham-treated controls.
Stilwell et al. 2008	Dairy calves 6 months	LA ¹ epidural, NSAID ³ SC + LA ¹ epidural, NSAID ² + LA ¹ epidural	Bu	5 min	Cortisol 6 and 24 hours significantly higher in non-treated castrated group. At 24 and 48 hours, epidural LA-carprofen-treated calves were first to arrive at the feed trough and had fewer pain-related behaviours.
Boesch et al. 2008	Dairy calves 1 week	LA ^{1,2,3}	Bu	20 min	Cortisol significantly lower in group lidocaine. Signs of pain were weak in general, but LA reduced them. No difference between lidocaine and bupivacaine.
Pieler et al. 2013	40 Fleckvieh calves	Xyl + LA ⁴	SR, Su, Bu, C	Xyl + LA ⁴ 5 min	With pretreatment, methods did not differ regarding stress/ pain at surgery. Increased locomotion and decreased lying with SR and Su.

Ref.	Animals	Analgesic protocol	Castration techniques	Prior medication	Outcomes
Durand et al. 2019	Sheep 12 months old	LA ^{1,3} , LA ¹ + NSAID ³	Bu	LA 10 min and NSAID immediately	High cortisol, SAA, fatty acids, glucose, haptoglobin for saline and LA. LA+NSAID was effective for 3 days. Reduced attention. Behavioural signs of pain apparent up to 32 h post-castration.
Marquette et al. 2021	Crossbred suckler beef calves, 2.5 and 5.0 months old		Bu, C		No effect on hematology, body temperature or growth. Older calves had higher cortisol, higher scrotal circumference. Older calves had more abnormal postures.

2.4.2. How to measure reindeer castration pain and discomfort?

Based on my experience as a veterinarian working with reindeer, at the immediate moment of clamp castration, bulls may wave their ears, their eyes can turn to show eye white, and they can struggle or kick and groan. Panting and defecating also occur. However, no studies exist on how acute castration pain alters reindeer behaviour.

2.4.1.1 Ethograms and grimace scales

Ethograms are a description of behaviours during a defined period. Data are collected either via direct observation or video recordings. The changes in behaviour are scored either numerically or proportionally and are analysed with available software. Ethograms are subjective and rely on the experience of the evaluator, and their weakness is also the possible effect of human presence on the behaviour (Tschoner, 2021). For example, increased respiratory rate may be an obvious sign of pain (Kata et al., 2014), but in reindeer increase in respiratory frequency may also reveal mental and physical stress and hyperthermia (Lian et al., 2019). Ethograms have been made for several species, mainly in post-castration pain studies (e.g. Robertson et al., 1994; Molony et al., 1995; Molony et al., 2002; Melches et al., 2007; Thüer et al., 2007, Medeiros do Nascimento et al., 2023), but neither during nor post castration pain ethograms have been yet published for reindeer. In such ethograms, abnormal postures, changes in appetite, increase or reduction in activity, restlessness, foot stamping, kicking, easing quarters, tail wagging, head turning, and vocalization are scored.

In recent years, identifying and scoring possible pain-specific behaviours using pain scales and facial expressions has been increasingly reported. Horse grimace scales using routine castration have been described by Dalla Costa et al. (2014). Changes in ear position, orbital tightening and some tension in the chewing muscles were largely similar as earlier reported for rodents and rabbits (Langford et al., 2010; Leach et al., 2012; Sotocinal et al., 2011; Keating et al., 2012). According to Gleerup et al. (2015b), equine pain face comprised alterations in ear positions (asymmetrical/low), eyes (angled, staring), nostrils (dilated) and muscle tension of the muzzle and cheeks, but not always simultaneously. In dairy cows, the Cow Pain Scale included attention towards the surroundings and response to approach, low head and ears and arched back positions, and tense facial expressions. Visible eye white was also inspected, but was not statistically relevant (Gleerup et al., 2015a). In sheep, pain was scored with tightened head muscles (orbital and masseter), abnormal ear position, lip and jaw profile and abnormal nostril and philtrum shape (McLennan et al., 2016).

2.4.1.2 Cortisol and other blood biomarkers

Nonspecific markers for emotional distress and tissue damage are measuring blood biomarkers, e.g. cortisol, substance P, and acute phase proteins or lymphocytes, as shown in Table 1. It is noteworthy that cortisol, in particular, as a stress hormone, is affected by e.g. handling, restraint and human presence (Tschoner, 2021). Stressful handling and tissue sampling may therefore confound the results (Weary et al., 2006), adding to various reasons that complicate the interpretation of physiological data in the assessment of animal welfare (Rushen, 1991).

It has been shown in reindeer that collecting many so-called objective markers, such as blood biomarkers, add to handling stress, and, for example, cortisol levels are expected to rise due to capture and restraint, as reviewed by Lian et al. (2019). Rehbinder and Nikander (1999) pointed out that the cortisol levels of differently treated reindeer rose significantly after 3 minutes, when the animals were first captured with a *suopunki/suohpan*, a special traditional rope thrown to catch reindeer, and held stationary. The rise in stress-induced metabolites, ASAT, urea and cortisol, following rough handling with a "lasso" was reported by Wiklund et al. (1996b). Blood sample collection has been shown to influence reindeer heartbeat frequency and plasma cortisol and noradrenalin concentrations (Säkkinen et al., 2004). The effect of capture, drugs, stress, season, and sample size should be carefully considered when interpreting hematology and serum biochemistry in reindeer (Miller et al., 2013). Even non-invasive sampling of feces with repetitive entries into the enclosure may trigger increased cortisol levels in reindeer (Carlsson et al., 2016; Özgün Gülzari et al., 2019). More chronic stress could be addressed by

measuring cortisol from hair samples (Sheriff et al., 2011), but it has been claimed that a single ACTH challenge does not increase hair cortisol in captive caribou and reindeer (Ashley et al., 2011).

2.4.1.3 Activity and changes in active behaviour

In recent years, numerous new tracking techniques have emerged that aid livestock management and enhance animal welfare (Herlin et al., 2021), and eliminate the human observer effect on behavioural study outcomes (Brown et al., 2013; Lyftingsmo, 2018). Objective and non-invasive assessment of activity and changes in active behaviour can be achieved using accelerometers or GPS trackers (Tschoner, 2021). Despite the advances in technical tools, observations may still be limited in detecting differences due to individual variation and social behaviours, for example, when one animal walks, others will follow (White et al., 2008).

Activity sensors can be used to quantify the total extent of movement: they can predict the active/inactive behaviour categories for reindeer with 99% accuracy, and the performance of activity loggers has been tested under ambient temperatures ranging from -30°C to $+20^{\circ}\text{C}$ (Van Oort et al., 2004). However, using the activity data for more detailed behavioural classification in free-ranging wild reindeer is challenging as the sensors often fail to separate lying and standing and identify more delicate minor behaviours (Lyftingsmo, 2016). Activity sensors are usually attached to a collar for reindeer because limbs are used for walking in deep snow and cratering. It is not easy to prevent the collars from turning around the neck because rigid attachment of the collar is a welfare concern for free-ranging animals. Fortunately, the requirement for rigid attachment is not necessary if the aim is to differentiate activity and rest only (Brown et al., 2013). Some new models are attached to the ear, but have, anecdotally, created some welfare concerns concerning ruptured ears.

Global Positioning System (GPS) collars can determine animal movements over large areas (Herlin et al., 2021), and the speed of the animals has proved to be a valid measure of activity (Ensing et al., 2014). The circadian rhythm and potential changes in movement patterns due to different circumstances may be revealed by analyzing the recorded GPS data from the locations (Herlin et al., 2021).

Whether reindeer have a weak circadian rhythm or not has been disputed (Van Oort et al. 2005, 2007; Mosser et al., 2014), but the latest research indicates that the diel rhythmicity with circadian master clock persists throughout the year, with ultradian bouts of activity and inactivity alternating across the 24-hour day, modified by the light-dark cycle (Bloch et al., 2013; Arnold et al., 2018). Reindeer in northern Norway displayed a significant rhythm of exactly 24 hours throughout autumn, winter and spring (Van Oort et al., 2005), and reindeer in Umeå, Sweden, had a polyphasic activity pattern with activity peaks occurring during both day and

night, with predominating diurnal activity, making reindeer essentially a diurnal animal (Eriksson et al., 1981). Activity levels vary with both biological and environmental parameters, with free-ranging caribous being more active than captive reindeer, due to foraging behaviour (Mosser et al., 2014), and activity increasing significantly in reindeer and caribou at the coldest winter temperatures with behavioural thermoregulation (Van Oort et al., 2007; Mosser et al., 2014).

Research knowledge on how reindeer castration pain and analgesia administration alter the daily activity patterns does not yet exist.

2.4.1.4 Temperature and other measurable objective means

Other tools for gauging pain perception include measuring feed intake, daily weight gain, temperature and heart rate (Tschoner, 2021). In freely grazing reindeer, the first two options are not feasible, and measuring daily weight gain under ambient conditions would impose significant stress on the study animals. Timisjärvi et al. (1979) studied 6-month-old reindeer calves and showed that the mean heart rate of a resting calf was 50–60 beats per minute, while various irritants, including pain and immobilization, led to rapid increases of up to 250 beats/min.

Reindeer have extremely thick and insulated winter hair, and they are therefore very susceptible to stress-induced body temperature increases (Lian et al., 2019). The normal body temperature for resting reindeer is 38.6 ± 0.2 °C (Blix et al., 2011), and rectal temperature may be lower than the core body temperature (Ozeki et al., 2014). Heat stress in reindeer is strongly associated with capture method, with additive effects from physiological and mental stressors, such as fear, metabolic heat from muscular exertion, anesthetic drugs, or high ambient temperature (Lian et al., 2019). Signs of heat stress, seen as panting, for example, begin at a brain temperature of 39.0 °C, whereas the threshold for selective brain cooling during exercise is elevated to 39.5 °C (Blix et al., 2011). The clinical point for hyperthermia is ≥ 2 °C greater than normal temperature (Arnemo et al., 2014). How castration pain and handling during castration affects reindeer body temperature has not yet been studied.

2.5 Castration pain management

Controversially, the painful procedure itself acts postoperatively partly as physical alleviation of the tissue damage pain, as the clamps destroy the innervation and therefore interrupt nerve impulse transmission from distal tissues, preventing pain impulses from the testes and parts of the scrotum, except from the uncrushed medial parts of the scrotum (Molony et al., 1993; Mellor and Stafford, 2000).

In practice, castration pain can be managed with local anesthesia, with or without sedation and/or narcosis, and postoperative pain is treated with nonsteroidal anti-inflammatories, peroral or injected. A review of the published literature suggests that multimodal analgesic regimens that extend into the postoperative period are more effective at mitigating pain and distress associated with castration than a single drug modality (Coetzee, 2011).

The medication of these semi-domesticated, free-ranging animals is confounded by the slowing of metabolism by even more than 50 % during the harsh winter months (Mesteig et al., 2000; Pösö, 2005; Arnold, 2020), affecting drug absorption and metabolism. Metabolism of xenobiotic compounds is generally slower in reindeer in wintertime, which needs to be considered when medicating reindeer (Laitinen et al., 1980 a,b, 1982 a,b; Juvonen et al., 1984).

2.5.1 Sedation and/or anesthesia

Even though general anesthesia would render an animal unconscious, it is considered impractical for use in large numbers of farm animals (Mellor and Stafford, 2000), and it is not possible to provide anesthesia gases and supplemental oxygen under field conditions. Moreover, sedating reindeer is problematic. Ruminants, in general, are difficult patients to anesthetize. Wild ruminants, which can describe modern-times reindeer– at least to some extent, with their tendency to suffer from a variety of stress-related complications, are exceptionally difficult to anesthetize (Caulkett and High, 2004). Wild animal species immobilized with either opioid or non-opioid-based combinations, often combined with alfa2-agonists, frequently demonstrate side-effects that can cause hypoxemia (Ryeng et al., 2001) and elevated fluid loss and therefore predispose anesthetized them to CM (Paterson, 2014). Estimating the dose–response is challenging, and these animals sedate too lightly or dangerously deeply (Soveri et al., 1999; Evans et al., 2013; Paterson, 2014). Sedatives slow the metabolism, weaken the thermoregulation and decrease the body temperature (Vasseur et al., 2010). Hypothermia slows the recovery, as the metabolism of sedatives is delayed at lower temperatures, and the risk of adverse effects is increased (Haskins et al., 2007). The fact that the reindeer castration procedure is mostly performed during the cold winter period adds to the risks mentioned because snow and rain, combined with wind, enhance heat loss (Arnemo et al., 2014).

Based on several studies considering reindeer anesthesia, medetomidine, also combined with ketamine, seems to be a good sedation agent for reindeer both in autumn and in late winter, and the effects of medetomidine can be effectively antagonized by atipamezole (Soveri et al., 1999; Ryeng et al., 2001; Arnemo and Aanes, 2009; Evans et al., 2013). Unfortunately, neither medetomidine nor atipamezole is registered for use in production animals in Finland, and there are no

other anti-sedative options available for reindeer (Finnish Food Authority list of medicines accepted for use for production animals). Moreover, Ranheim et al. (1997) showed that reindeer became resedated 0.5–1 h after reversing medetomidine anesthesia with atipamezole, which makes its use even more problematic.

2.5.2 Local anesthesia

Local anesthetic compounds provide analgesia by producing a reversible loss of sensation in a localized area, by blocking open sodium channels of nerve cells, preventing the generation and transmission of nerve (pain) impulses (Coetzee, 2011). Local anesthesia (LA) reduces the level of indicators of acute castration pain (Stafford and Mellor, 2005; Thüer et al., 2007; Melches et al., 2007; Mellema et al., 2006), however, several studies on bovine calves have shown that LA did not allow pain-free castration using the Burdizzo clamp method (Fisher et al., 1996; Stafford et al., 2002; Ting et al., 2003; Thüer et al., 2007; Boesch et al., 2008). Durand et al. (2019) showed in 12-month-old texel sheep that LA was only partially effective at relieving pain, and only during its 2 hours of activity, while the combination of LA and NSAID was effective for up to 3 days post-castration. Similar results in lambs were reported by Mellema et al. (2006).

Moreover, the short duration LA products available for food-producing animals in the Finnish market, i.e. procaine hydrochloride and lidocaine, do not provide longer term alleviation for postoperative pain (Graf and Senn, 1999), even though there is evidence of LA being beneficial during the first few hours after Burdizzo castration in lambs (Mellema et al., 2006). Thus, a combination of LA and NSAIDs is more effective than LA alone (Stafford and Mellor, 2005; Coetzee, 2011; Durand et al., 2019).

Local anesthesia can be administered either locally on the spermatic cord and under the skin, or epidurally (Currah et al., 2009). The most effective injection sites are the neck of the scrotum, deposited into the vaginal cavity of the scrotum, or both testes, i.e. injecting LA only into the spermatic cords is less effective (Mellor and Stafford, 2000). Lidocaine has 90 minutes duration of action. Epidural anesthesia provides regional analgesia, and the effect is expected 5 minutes after the administration, lasting 10–115 minutes (Coetzee, 2013). If the reindeer's ability to use the hind limbs is suppressed for this long, the technique is too complicated to be used in free-ranging animals that are often released into the wild after the procedure.

The efficiency of LA depends on the volume used (Currah et al., 2009) and the waiting time. Unlike slow-acting procaine hydrochloride (VetViva Richter GmbH, 2013), the onset for lidocaine is quite rapid, 2–5 minutes, but the waiting time used in different calve castration trials was 5–20 minutes after injecting local anesthesia,

before castration with Burdizzo clamps (Coetzee, 2011). Mellor and Stafford (2000) reviewed that local anesthetic may be delivered 10–20 min before treatment, and Thüer et al. (2007) suspected 5 minutes waiting time to be too short for adequate analgesia.

If the castration method includes increasing stress because of a long holding time for the animal, perhaps struggling, waiting for the effect of local anesthesia, it can, in the worst-case scenario, lead to a more serious animal welfare problem, even if the intention was to minimize pain and stress (Jordbruksverket, 2008). The injection of lidocaine may be painful or irritating itself (Stafford and Mellor, 2005), and the injection-associated pain can be decreased with sodium bicarbonate at a ratio 1:10 immediately before use (Coetzee, 2013). Boesch et al. (2008), on the other hand, considered irritation to be minimal. Lidocaine should be used with caution due to toxicity – the total dose should not exceed 5 mg/kg for small ruminants (Coetzee, 2013), or less than 4 mg/kg for IV regional anesthesia, without epinephrine (Caulkett and High, 2004). However, there has been no research on the effect of LA on reindeer castration.

2.5.3 Analgesia with NSAIDs

NSAIDs are medicines that help reduce inflammation and pain. NSAIDs block the action of two forms of cyclooxygenase enzyme: COX-1 and COX-2. By inhibiting COX, NSAIDs reduce the production of prostaglandins. Prostaglandins promote inflammation, pain, and fever, thereby NSAIDs lower inflammation, reduce the sensation of pain, and decrease body temperature. Of the two enzymes, COX-1 is expressed faster, so that NSAIDs with COX-1 activity provide more immediate pain alleviation, but also represent a higher risk for adverse effects in the gastrointestinal tract and kidneys than more selective COX-2 inhibitors (Coetzee, 2013).

Veterinarians routinely use off-label analgesia on reindeer to treat various inflammatory conditions and injuries (Pizzi et al., 2011; Gonzales-Alonso-Alegre et al., 2013; Monticelli et al., 2017, Laaksonen, 2019). Currently, no analgesic drugs have been approved for the European market for reindeer. However, according to cascade rules in Europe, the use of drugs approved for other production animal species is accepted (Directive 2019/6/EU articles 112–115). The dosages in reindeer practice are extrapolated from those for other ruminant species because there is no published information concerning the pharmacokinetics of any NSAIDs in reindeer.

The onset of analgesic activity of NSAIDs is slow; and in most research NSAIDs have been administered at least 20 minutes before a painful procedure to ensure sufficient tissue concentrations for analgesic effects (Coetzee, 2011). This poses a challenge when dealing with reindeer subjected to repeated capture or prolonged restraint. Moreover, NSAID alone is not sufficiently effective to reduce the acute

pain impulse barrages associated with the initial tissue injury of bovine calves (Mellor and Stafford, 2000; Stafford and Mellor, 2005; Coetzee, 2011). The role of NSAIDs is merely important in treating postoperative pain, i.e. their main action is targeted towards inflammation-related pain, so that little or no alleviation of the pain caused by clamping itself can be expected (Mellor and Stafford, 2000).

2.5.3.1 Meloxicam

Meloxicam is one of the NSAIDs used in reindeer practice, for alleviating pain and treating various inflammatory processes (Laaksonen, 2019). It is a COX-2 selective prostaglandin E₂ inhibitor with anti-inflammatory, antipyretic and analgesic properties. Meloxicam has been on the market since the 1990s and is accepted for use in cattle, pigs and horses. Bioavailability is high after subcutaneous injection in cattle (mean 92%), and meloxicam absorbs well after oral administration, with good bioavailability and a relatively long half-life (24–28 hours in calves). Tolerance studies performed in cattle show that meloxicam is well tolerated at 0.5–1.5 mg/kg for 5 days. The main side effect of meloxicam is ulcerogenic activity in the gastrointestinal tract (EMA, 2010), which is important to remember with reindeer, a species prone to gastric ulcers caused by handling and herding stress (Rehbinder, 1982). Doses in reindeer, again, are extrapolated from those for other species. Only a few case reports describe the use of meloxicam in small numbers of reindeer (Pizzi et al., 2011; Gonzales-Alonso-Alegre et al., 2013; Monticelli et al., 2017).

However, the therapeutic dose and pharmacokinetics of meloxicam vary significantly according to dosing routes and species (Busch et al., 1998; Sinclair et al., 2006; Kimble et al., 2013; Mendoza et al., 2019), even among different ruminant species (Shukla et al., 2007; Coetzee et al., 2009; Ingvast-Larsson et al., 2011; Kreuder et al., 2012; Stock et al., 2013; Karademir et al., 2016; Woodland et al., 2019; Hempstead et al., 2020), or ruminant age status (Coetzee et al., 2012; Mosher et al., 2012; Jokela et al., 2023), or even lactation period stage in cows (Gorden et al., 2018; Shock et al., 2019). Producer-friendly oral administration of meloxicam has been shown to alleviate some of the inflammation and stress following surgical castration of weaning bull calves (Brown et al., 2015). Even with repeated oral dosing, meloxicam withdraws reasonably rapidly, in 21 days in beef calves (Coetzee et al., 2015). Therapeutic levels of meloxicam vary markedly from a horse's 0.13–0.2 µg/ml (Toutain and Cester, 2004) to a dog's 0.82 µg/ml (Montoya et al., 2004) to 0.57–0.93 µg/ml in human plasma (Türck et al., 1996). Therefore, extrapolating doses is not good practice, and information is not available on pharmacokinetics of meloxicam in reindeer, especially during wintertime, when medication is usually needed.

3 Aims of the study

The main aims of this study were to define pain behaviours and investigate the best current pain alleviation practices in reindeer castration.

The specific aims were:

1. To investigate the wintertime pharmacokinetics of the non-steroidal anti-inflammatory drug meloxicam in reindeer after intravenous and oral dosing.
2. To investigate the effects of castration on reindeer behaviour, rectal temperature and respiratory rate during the clamp castration procedure, and document determine how these are affected by handling, local anaesthesia and a non-steroidal anti-inflammatory drug.
3. To investigate the effects of clamp castration on activity and daily rhythm of reindeer during the first hours up to three days post procedure and determine how these are affected by a non-steroidal anti-inflammatory drug.

The main hypotheses for the studies were:

During clamp castration:

1. Reindeer show pain-related behaviours indicating that they feel pain.
2. Local anesthesia lessens the behavioural signs of pain.
3. Perioperatively administered NSAID does not affect the behaviour at the moment of clamps crushing the tissue.
4. Prolonged handling adds to stress, shown as increased body temperature.

and

5. Post clamp castration: Untreated pain affects the activity patterns of reindeer.

4 Materials and methods

This thesis is based on three experiments (partial crossover study on meloxicam pharmacokinetics and two clinical trials) reported in three original scientific peer-reviewed articles (I–III). The main methods for study designs, animals and their care and data collection and analyses are provided in this section. The original articles, with more detailed descriptions, are included at the end of this thesis.

The studies were approved by the Project Authorization Board (ESAVI/36846/2019) (I–II), the University of Helsinki Viikki Campus Research Ethics Committee (Statement 16/2020) (III) and the Finnish Medicines Agency (Vetkl-nro 04/2019) (I–III), and conducted at the University of Helsinki reindeer research site, located in Savukoski, Lapland, Finland, and in Naltio round-up enclosure (67°N 29°E), managed by the Kemin-Sompio reindeer herding cooperative.

The Kemin-Sompio reindeer herding cooperative is the biggest cooperative in Finland, with a maximum of 12 000 live reindeer after slaughter selection, located in central and eastern Lapland, i.e. in the area specifically intended for reindeer husbandry, and bordering Russia. Kemin-Sompio partly covers the areas of Savukoski and Sodankylä municipalities, former the Keminkylä and Sompio Lapp villages. Of the Kemin-Sompio reindeer, 2/3 graze freely year-round in the great wilderness areas of northern Savukoski, and are gathered from the wild twice a year, in the summer for calf marking and in the fall and winter for round-ups. The young males leased to the studies I and II were not mainly earmarked as calves and had been grazing freely in the wilderness since birth. The adult males used in the study III were year-round freely grazing bulls and were therefore less used to human presence.

4.1 Study designs

We chose and marked the reindeer for the studies I–II in round-ups in October, but released them to the wild again, to the summer side of the grazing area. After the rut was over and snow stayed on the ground in November, we started capturing those marked study animals from the round-ups held in November and December and transferred them by van from various round-up locations to the research site, 14 km north from the Savukoski village center.

The objective of the study I was to investigate the pharmacokinetics of orally and intravenously (IV) administered meloxicam. We used a crossover design with an 11-day washout period. A total of eight young male reindeer, aged 1.5–2.5 years and weighing 74.3 ± 6.3 kg, mean $\pm$ standard deviation, were enrolled. The reindeer were administered meloxicam (0.5 mg/kg IV or orally, Metacam 20 mg/ml inject., Boehringer Ingelheim Vetmedica GmbH, Germany, and Metacam, 15 mg/ml, oral suspension for horses; Boehringer Ingelheim Vetmedica GmbH). Blood samples were repeatedly collected from the jugular vein for up to 72 hours post administration. Plasma samples were analysed for meloxicam concentrations with ultraperformance liquid chromatography combined with triple quadrupole mass spectrometry. Noncompartmental analysis for determination of pharmacokinetic variables was performed. See detailed report in article I.

The objective of the study II was to investigate the acute pain behaviours and rectal temperatures of castrated reindeer and how handling, LA and NSAID affected them. A total of 55 reindeer were randomly allocated to five different treatment groups, $n = 11$ in each. The treatment groups were: 1) traditional castration (TRAD), 2) castration with meloxicam (NSAID), 3) castration with lidocaine (LA), 4) castration with meloxicam and lidocaine (LA+NSAID), and 5) non-castrated control (CONTROL). The target handling duration was one minute for groups 1 and 2 (short treatment), and six minutes for groups 3–5 (long treatment), recorded with a stopwatch. In the CONTROL group, the same animal served as a control for both short and long treatments. Groups 2–4 were medicated and 1–4 castrated. The NSAID and LA+NSAID groups were administered a 2.5 ml subcutaneous injection of meloxicam (Metacam 20 mg/ml inject., Boehringer Ingelheim Vetmedica GmbH, Germany, with a target dose of 0.5–0.7 mg/kg) on their necks while still in a standing position, just before they were pushed over. The LA and LA+NSAID groups were treated with 10 ml lidocaine (Lidor vet. 20 mg/ml inject., Richter Pharma AG, Austria) injected and distributed to their spermatic cord and scrotal neck. Rectal temperature was measured (Beurer FT15/1 instant thermometer, Germany) 2–4 times depending on the treatment length. The behaviour was continuously filmed and scored with Cowlog (Pastell, 2016) after an ethogram was created. See detailed report in article II.

The castrated animals were slaughtered at the abattoir in Rovaniemi after the 28-day withdrawal period required for the drugs used. The reindeer were moved gently from the research site enclosures to a professional carrier's transport vehicle, a lasso was not used for handling. The transport distance was 193 km. After slaughtering, the abomasums were inspected for ulcers by two veterinarians experienced in reindeer meat inspection.

The objective of the study III was to investigate the postoperative castration pain and how it affects daily rhythms and activity, and whether NSAID changes the behaviour. The study was performed during the Kemin-Sompio cooperative round-

up in Naltio, Savukoski, October 5th, 2020. We attached an Ultracom GPS tracker to 16 healthy reindeer that were, as a standard herding procedure, castrated at about 5 years of age, and their approximate weights were between 130 and 160 kgs. The selected animals were voluntarily leased to our study. Half of the reindeer were castrated traditionally with emasculator clamps, without analgesia. The remainder were administered a subcutaneous NSAID injection of 4 ml meloxicam (Metacam 20 mg/ml inject., Boehringer Ingelheim Vetmedica GmbH, Germany) on their neck, in addition to traditional castration. All animals were administered ivermectin at 0.2 mg/kg live weight subcutaneously (Virbamec 10 mg/ml, Virbac, France). The reindeer were released from the round-up corral to the wild and followed with GPS trackers 3 weeks post procedure. A detailed report is given in article III.

4.2 Animals, housing, feeding and instrumentation

Our inclusion criteria in all three experiments were healthy, intact male reindeer with no signs of clinical disease, for the first two studies two weeks before the experiments, and a reindeer was excluded if it showed any signs of disease. A summary account of animals, housing, feeding and instrumentation is presented in Table 2. For more detailed care and conditions, see details in articles I, II and III.

Table 2 Animals, housing, feeding and instrumentation in studies I–III.

	Crossover	Clinical trial 1	Clinical trial 2
No of reindeer	8	55	16
Age	1.5–2.5 years	1.5–2.5 years	5+ years
Feed	Commercial Kinnusen Mylly Poro Balans 2, dry hay, lichen, snow ad libitum	Commercial Kinnusen Mylly Poro Balans 2, dry hay, lichen, snow ad libitum	Natural pastures
Site	HU reindeer research site	HU reindeer research site	Naltio round-up corral/wilderness
Time	Feb 2020	Dec-Jan 2019–2020	Oct 2020
Instrumentation	Blood sample analysis	Behavioural analysis Rectal temp.	GPS tracker analysis
Article	I	II	III

4.2.1 Blood samples for meloxicam concentration analysis (I)

Blood samples (4 ml EDTA tubes) were systematically collected from the jugular vein of reindeer at specified intervals following meloxicam administration. For the intravenous (IV) treatment, samples were taken at 1, 3, 6, 12, 24, 36, 48, and 72 hours, while for the oral treatment, samples were collected at 3, 6, 12, 24, 36, 48, and 72 hours. During sample collection, reindeer were restrained with a halter and rope but were subsequently released into a 5-hectare enclosure for an 11-day washout and withdrawal period.

Following the washout period, the reindeer were recaptured a day before the second phase, with a baseline blood sample obtained during the clinical examination to assess meloxicam plasma concentration and confirm the adequacy of the washout period. The second phase was performed according to the crossover design, animals first in the IV treatment now assigned to the oral treatment, and vice versa.

Blood samples were stored on snow, and the plasma was separated by centrifugation for 10 minutes at 1509 g immediately after blood was collected from all eight reindeer. Afterwards, the plasma was stored at -20 °C until analysed.

A quantitative analysis of meloxicam was performed with ultraperformance liquid chromatography combined with triple quadrupole mass spectrometry (Waters Acquity UPLC + Waters Xevo TQ-S triple quadrupole MS, Waters Corp., MA, USA) with a reversed-phase column (Waters Acquity HSS T3, 1.8 µm, 2.1 × 30 mm; Waters Corp.). For more details, please see article I.

4.2.2 Rectal temperatures (II)

Rectal temperature (T) was measured (Beurer FT15/1 instant thermometer, Germany) from all animals immediately after pushing over (To) and after castration/before releasing; short treatments TRAD and NSAID at one minute point (T1); long treatments LA, LA+NSAID and CONTROL at 6-minute point (T6). In addition, rectal temperature was taken from LA, LA+NSAID and CONTROL at one minute point after LA was injected (T1), and in the middle of holding (3-minute point, T3). Refer to Figures 4 and 5 and article II for more detailed protocols.

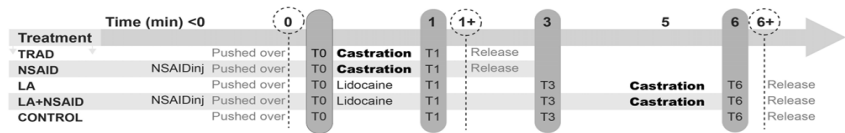


Figure 4 Rectal temperature measurement protocols. TRAD = traditional castration, NSAID = castration with meloxicam, LA = castration with lidocaine, LA+NSAID = castration with meloxicam and lidocaine, and CONTROL = non-castrated control. To, T1, T3 and T6 are the rectal temperature measurement points.

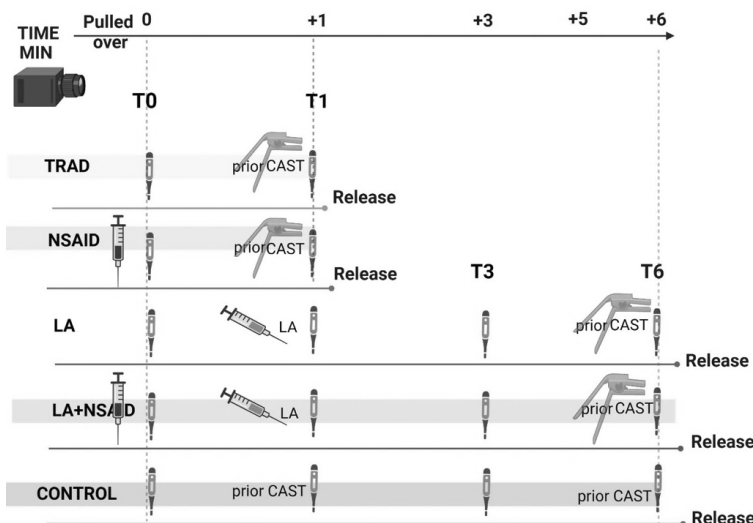


Figure 5 Procedure protocol for treatments and control. TRAD = traditional castration, NSAID = castration with meloxicam, LA = castration with lidocaine, LA+NSAID = castration with meloxicam and lidocaine, and CONTROL = non-castrated control. Black syringe indicates the injection of NSAID, grey syringe indicates the injection of LA. Clamps indicate the moment of castration for short and long treatments. Thermometer indicates the temperature measurement points T0, T1, T3 and T6. Prior and CAST indicate the behaviour observation periods for all the treatments and control. ©Biorender

4.2.3 Behavioural assessment: video recording and creating an ethogram (II)

The behaviour of each reindeer was recorded continuously during the castration procedure (Canon 5D MKIII camera from Canon Inc., Japan). Due to the limited daylight hours during the polar night at the end of December, we supplemented the lighting in the trial enclosure with three additional spotlights (Velvet Light 1 from Velvet, Spain; Nordlight LED 2400 from Blue Import AB, China; and LED Energie Prof line 850 from LED Energy Nordic AB, Sweden).

Given the absence of prior research on pain behaviours in reindeer, we created an ethogram as a key component of this study. The initial framework of the ethogram was built by outlining the behaviours from ethograms created for other animal species (Molony et al., 1995; Dawkins, 2007). Subsequently, two experienced ethologists, two veterinarians, and a veterinary student - the last three all well-versed in reindeer herding - identified relevant pain-related behaviours, ensuring their expression and visibility through careful examination of video samples. Facial expressions, except for nostril movements, were omitted from the final ethogram due to limitations in video quality for scoring such delicate movements in surrounding conditions of sleet, snow, and polar night. The included

behaviours comprised head, neck, and leg movements, respiratory rate, panting, nostril closure, defecation, and abdominal muscle contraction (refer to Table 3).

The videos were then scored continuously with CowLog 3 (Pastell, 2016) in a randomized order by a single researcher who was unaware of the treatments. The reindeer behaviours were scored from observation periods prior to and during castration and LA injection, individually set according to castration durability. Median castration duration 24.6 seconds was used in CONTROL, respectively (Figure 5). For all observation periods, the number of bouts was counted for single kicks, leg flexions and straightenings, defecating and single head movements. The number of bouts, total duration of bouts, and mean bout lengths were assessed for the remaining behaviours. Respiration rate was also counted in 15-second sequences during the measurements of To–T6, LA injection, and castration. For further detailed information on behavioural analysis, see article II.

Table 3 Definitions for behaviours used for scoring clamp-castrated reindeer treated with a local anesthesia and/or meloxicam, traditionally castrated reindeer, and the holding control.

Behaviour	Description
Defecating	Feces coming out of rectum.
Single kick	Sudden angled, forward, or backward movement of a single leg flexing and straightening, max 1/s.
Several rapid kicks more than once/s, struggling	Movement of one or more legs in a struggling manner.
Leg flexion	Leg is bent close to the corpus, not kicking.
Leg straightening	Leg is straightened, over 45 degrees, not kicking.
Neck flexion	Neck is curved and muzzle is turned towards the sternum, not lifting head off the ground.
Neck straightening	Neck is straightened and muzzle is directed forward, not lifting head off the ground.
Head lifting (off the ground)	Upper part of neck, i.e. cheek and upper neck muscles, are lifted off the ground.
Other head movements	Minor changes in head position, head is not lifted off the ground
Head movement attempt	Restrained animal tries to move its head, which is visible as a contraction of the neck muscles and/or as movements by the holder.
Nostril closure	Nostrils are closed and form a line, lasting a minimum of 2 seconds.
Panting	Breathing heavily with mouth open and rhythmical movements of head and/or chest.
Abdominal muscle contraction	Abdominal muscles retreat towards the spine while no respiration movements are visible.

4.2.4. Activity assessment with GPS trackers (III)

Ultracom GPS trackers (Tracker Ltd., Oulu, Finland), attached to a collar, were preset to give the location twice per hour during the three weeks after the trial. GPS data were then transferred into geographical map points.

The distances between two sequential measurement points were analysed using the formula (L1= latitude 1, L2= latitude 2, D1= longitude 1 and D2 = longitude 2):

$$\text{ACOS}(\text{COS}(\text{RADIANS}(90 - \text{L1})) * \text{COS}(\text{RADIANS}(90 - \text{L2})) + \text{SIN}(\text{RADIANS}(90 - \text{L1})) * \text{SIN}(\text{RADIANS}(90 - \text{L2})) * \text{COS}(\text{RADIANS}(\text{D1} - \text{D2}))) * 6371$$

4.3 Data analysis

4.3.1 Noncompartmental analysis of meloxicam (I)

Noncompartmental analysis was used to calculate the following pharmacokinetic variables from the concentration-time-dose data (WinNonlin 5.2; Certara, NJ, USA; Linear Trapezoidal LinearLog Interpolation, uniform weighting): area under curve (AUC), maximum serum concentration ($C_{\max}$), time to maximum serum concentration ($t_{\max}$), mean residence time (MRT), clearance (CL), and volume of distribution at the steady state (V_{ss}), last two variables for the IV route only.

Elimination half-life was calculated with the formula ($\ln 2$ = the natural logarithm of 2):

$$t_{1/2} = \ln 2 \times \text{MRT}$$

4.3.2 Statistical analysis (II-III)

In study II, nonparametric statistical procedures were applied because most of the data were not distributed normally. The differences between the treatments in behaviours, respiratory rates and rectal temperatures were analysed with Kruskal-Wallis tests using pair-wise post-hoc comparisons. Friedman one-way repeated measure analysis of variance by rank with pair-wise post-hoc tests and Wilcoxon pair-wise tests were used to study the differences between sampling intervals for respiratory rates, temperatures and behaviours (prior and during castration) in short and long treatments, respectively. We scored the number of reindeer that reached the rectal temperature of at least 39.5 °C in To-T6 and analysed the differences between treatments with chi-square test analyses with Bonferroni corrections for multiple tests to adjust significant values for all pairwise comparisons. Spearman's rank correlations were computed to assess the relationship between T and respiratory rates. Details in article II.

In study III, before analyzing the data, we used a Bayesian statistical method to insert missing data (Chen and Åstebro, 2004). From the data we calculated mean hourly distances the reindeer travelled during the three days after castration, and the mean overall distance travelled each day, as well as the differences in diurnal activity, separating day (7:00-17:00) and night (17:00-7:00). As the data did not conform to a normal distribution, we analysed the differences between the treatments with a generalized estimating equation model (GEE), suitable for handling repeated measures data. See details of the models used in article III.

The statistical analyses were conducted using IBM SPSS Statistics (version 27.0 (II) and 29.0 (III), Armonk, NY, USA). P-values less than 0.05 were considered significant.

5 Results

5.1 Missing data

Study I: Three reindeer in the IV treatment were excluded from the analysis due to incomplete IV administration, and one reindeer due to an injury in the hind leg, so that complete series of blood samples were finally analysed from four animals in the IV treatment. Data were missing (laboratory error) at 6 hours post administration in one reindeer for the oral treatment, and all data from this animal were excluded from analysis, so that seven reindeer were left in the oral treatment analysis.

Study II: Due to technical problems, we excluded one reindeer from rectal temperature analysis from TRAD group, and one reindeer from behavioural and respiratory analysis from CONTROL group. Due to poor visibility caused by sleet, we were unable to calculate the respiratory rates for 9/54 animals at To (3 CONTROL, 2 LA+NSAID, and 4 TRAD), 8/54 animals at T1 (2 from both CONTROL and NSAID, 1 LA, and 3 LA+NSAID), 3/33 animals at T3 (2 CONTROL and 1 LA+NSAID), and 4/33 animals at T6 (2 from both CONTROL and LA+NSAID).

Study III: Three of the GPS reindeer were out of satellite reach for 1-2 hours during the first day and two reindeer 1-2 hours on days 2 and 3. From day 4 to day 6 four of the reindeer had 2-12 hours of missing data and on day 7 nine reindeer were out of reach. The first 3 days' data were therefore analysed.

5.2 Meloxicam pharmacokinetics (I)

Orally administered meloxicam was absorbed reasonably fast, with $t_{\max}$ in 6 hours, and had a long plasma mean half-life of 19.3 (16.7–20.5) hours. No evident adverse effects were detected after either treatment. Bioavailability (F) calculated for the three reindeer with complete data for both treatments was 49 (46–73) %, median (range). Observed mean $\pm$ standard deviation meloxicam concentrations in reindeer plasma versus time (in hours) after a single dose of 0.5 mg/kg administered IV ($n = 4$) and orally ($n = 7$) are graphically displayed in Figure 6.

Respectively, the calculated noncompartmental pharmacokinetic parameters following the IV and oral routes of meloxicam (0.5 mg/kg) administration are presented in Table 4.

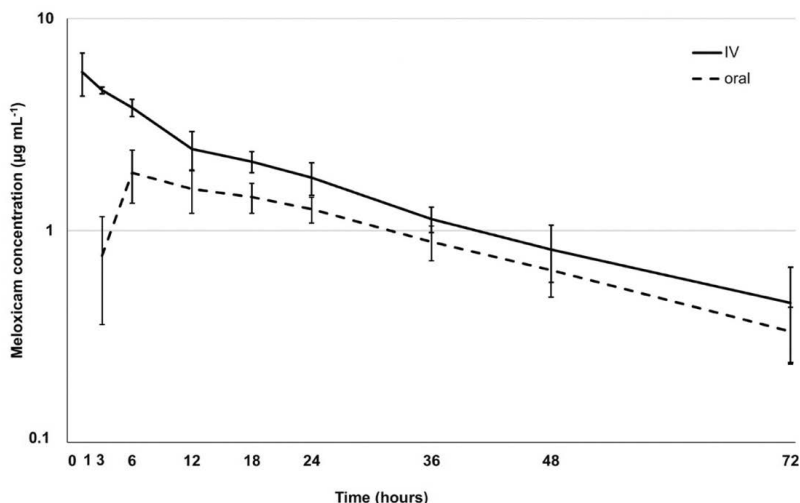


Figure 6 Mean $\pm$ standard deviation plasma meloxicam concentrations (in a half-logarithmic scale) in semidomesticated reindeer after administration of single doses (0.5 mg/kg) of oral ($n = 7$) and intravenous ($n = 4$) meloxicam.

Table 4 Median (range) values for pharmacokinetic parameters of meloxicam after administration of a single dose (0.5 mg/kg) to semidomesticated reindeer through intravenous (IV) ($n = 4$) and oral ($n = 7$) routes.

AUClast = area under the concentration-time curve from dosing (time 0) to the time of the last measured concentration, CL = clearance, Cmax = maximum plasma concentration, MRTlast = mean residence time, na = not determined, $t_{1/2}$ = elimination half-life, tmax = time to maximum plasma concentration, Vss = volume of distribution at steady state.

Parameters	Oral treatment	IV treatment
$t_{1/2}$ (hours)	19.3 (16.7–20.5)	15.2 (13.7–16.8)
tmax (hours)	6 (6–6)	na
Cmax ($\mu\text{g mL}^{-1}$)	1.82 (1.17–2.78)	na
AUClast (hour* $\mu\text{g mL}^{-1}$)	62.4 (51.5–88.6)	130.7 (102.9–876.0)
MRTlast (hours)	27.8 (24.1–29.6)	22.0 (19.8–24.3)
CL ($\text{mL}^{-1} \text{ hour}^{-1} \text{ kg}^{-1}$)	na	3.98 (2.63–5.29)
Vss (mL kg^{-1})	na	133 (113–151)

5.3 Effects of castration, pain alleviation and handling on rectal temperatures (II)

A total of 25 animals reached a rectal temperature of 39.5 °C. Of these, 2/10 were TRAD, 3/11 NSAID, 4/11 LA, 11/11 LA+NSAID, and 5/11 CONTROL. The number of LA+NSAID animals with $T \geq 39.5$ °C tended to differ from LA at T1 (54.5% vs 0%, $p = 0.05$) and differed from LA and CONTROL at T3 (100% vs 18.2% and 27.3%, respectively, $p < 0.001$) and from LA at T6 (90.9 % vs 36.4 %, $p < 0.02$). Five animals (all from LA+NSAID) reached a body temperature of at least 40.0 °C at T6, the highest measured value being 40.5 °C. One individual from the NSAID group reached 40.0 °C at T0.

Rectal temperature values did not show differences between the treatment groups at T0, T1, and T6. However, we found a difference between the treatments at T3 ($H(2) = 13.78$, $p = 0.001$): rectal temperature was higher in the LA+NSAID group than in the LA or CONTROL groups ($p = 0.002$). Post-castration temperature was significantly different between long (T6) and short (T1) treatments ($H(3) = 11.30$, $p = 0.01$); LA+NSAID (= T6) was higher than TRAD and NSAID (= T1) ($p < 0.03$). No other differences between the groups were established (Figure 7).

Overall, within each treatment, rectal temperatures increased significantly from T0 onwards. There were significant changes between the first and last measures: In the short treatments the temperature increased significantly between T0 and T1 for both TRAD ($z = 2.13$, $p = 0.03$) and NSAID ($z = 2.53$, $p = 0.01$), and the increase between T0 and T6 was significant for all the long treatments: LA+NSAID, LA, and CONTROL ($\chi^2(3) = 20.04$, $\chi^2(3) = 14.58$, $\chi^2(3) = 17.14$, $p < 0.02$ for all, Figure 7).

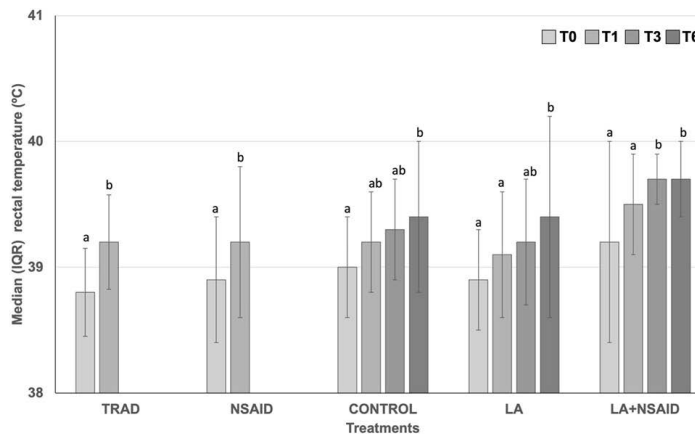


Figure 7 Rectal temperatures measured 2 and 4 times (during short and long treatments, respectively) from 54 reindeer, aged approximately 1.5 years, during different treatments: traditional castration (TRAD), castration with meloxicam (NSAID), castration with lidocaine local anesthesia (LA), castration with lidocaine and meloxicam (LA+NSAID) and holding control (CONTROL). Different letters (a, b) mark statistically significant differences between the measurement points within a treatment.

5.4 Effects of castration, pain alleviation and handling on behaviours and respiratory rate (II)

Panting and defecation were seen occasionally throughout the treatments. We observed very few of the behaviours included in the ethogram prior to castration. Abdominal muscle contractions were not observed in any of the treatments prior to castration, and nostril closures were only occasional. We did not register differences prior to castration between short or long treatments, or when comparing treatments without CONTROL.

However, during castration treatment differences were recorded in the numbers, bout durations, and total durations of nostril closures in both the short ($H(2) = 20.27$, $H(2) = 17.87$, $H(2) = 17.87$, respectively, $p < 0.001$ for all) and long treatments ($H(2) = 11.45$, $p = 0.003$, $H(2) = 10.99$, $H(2) = 10.97$, respectively, $p = 0.004$ for all): the number and duration of nostril closures were smaller in CONTROL (short) than TRAD or NSAID, and in CONTROL (long) than LA and LA+NSAID ($p < 0.05$ for all) (Figure 8). Similarly, there were treatment differences in the numbers, bout durations, and total durations of abdominal contractions, both in the short ($H(2) = 29.18$, $H(2) = 20.74$, and $H(2) = 20.74$, respectively, $p < 0.001$ for all) and long treatments ($H(2) = 21.09$, $H(2) = 19.47$, and $H(2) = 19.35$, $p < 0.001$ for all, respectively): the number and duration of abdominal contractions were greater in TRAD and NSAID than CONTROL (short), and in LA, and LA+NSAID compared with CONTROL (long), respectively ($p < 0.05$ for all) (Figure 9). During the local anesthesia procedure (i.e. handling the testicles and injecting lidocaine), neither abdominal muscle contractions nor nostril closures were observed.

The number of leg flexions during castration differed between short treatments ($H(2) = 6.19$, $p = 0.045$): TRAD showed a higher number than NSAID ($p = 0.046$), while CONTROL did not differ from either. Also, between the long treatments, the number of leg flexions differed ($H(2) = 9.49$, $p = 0.01$): CONTROL showed fewer leg flexions than LA ($p < 0.05$) and tended to show fewer flexions than LA+NSAID ($p = 0.05$). When comparing intervals prior to and during castration within the long treatments, the number of leg flexions increased in LA ($z = 2.46$, $p = 0.014$). In addition, during castration, the number of leg straightenings also increased for LA compared with prior to castration ($z = 2.07$, $p < 0.05$). No differences were observed within short treatments.

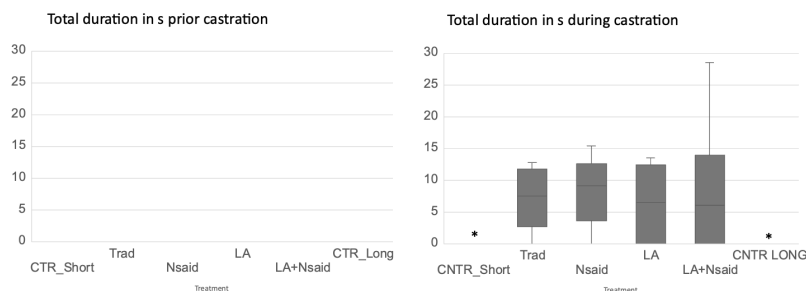


Figure 8 Total durations of nostril closures prior and during castration. Treatment groups were traditional castration (TRAD), castration with meloxicam (NSAID), castration with lidocaine local anesthesia (LA), castration with lidocaine and meloxicam (LA+NSAID) and holding control (CONTROL), n = 11 in each. In the CONTROL group the same animal served as a control for both short and long treatments.

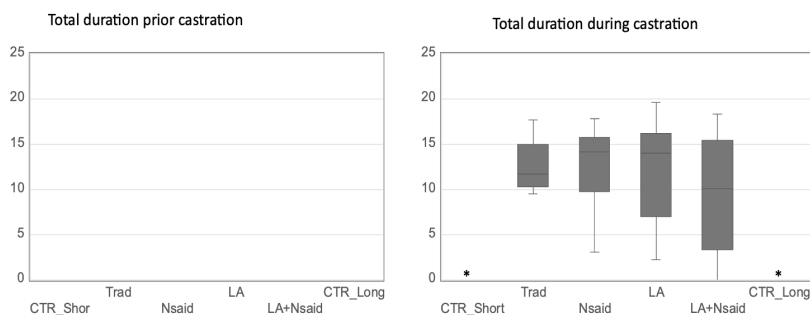


Figure 9 Total durations of abdominal muscle contractions prior and during castration. Treatment groups were traditional castration (TRAD), castration with meloxicam (NSAID), castration with lidocaine local anesthesia (LA), castration with lidocaine and meloxicam (LA+NSAID) and holding control (CONTROL), n = 11 in each. In the CONTROL group the same animal served as a control for both short and long treatments.

The median respiratory rates decreased overall during the experiment within all treatments. Between treatments, the respiratory rates differed at T1 ($H(4) = 16.56$, $p = 0.002$): LA+NSAID being higher than NSAID ($p = 0.005$), TRAD ($p = 0.03$), and LA ($p = 0.02$). Respiratory rates also differed at T3 ($H(2) = 9.41$, $p = 0.009$): they were higher for LA+NSAID than LA ($p = 0.03$). No other differences were recorded (Figure 10). The median (IQR) respiratory rates during injection of a local anesthesia were 36(16) for LA ($n = 11$) and 44(26) for LA+NSAID ($n = 9$), not differing from T0 or T1.

Overall, when comparing the treatments without CONTROL during castration, the respiratory rates differed ($H(3) = 10.53$, $p = 0.015$): TRAD had the highest median respiratory rate differing from LA (38(40) vs 32(20) breaths per minute, $n = 10$ and 11, respectively, $p = 0.024$). For more detailed results on respiratory rates see article II.

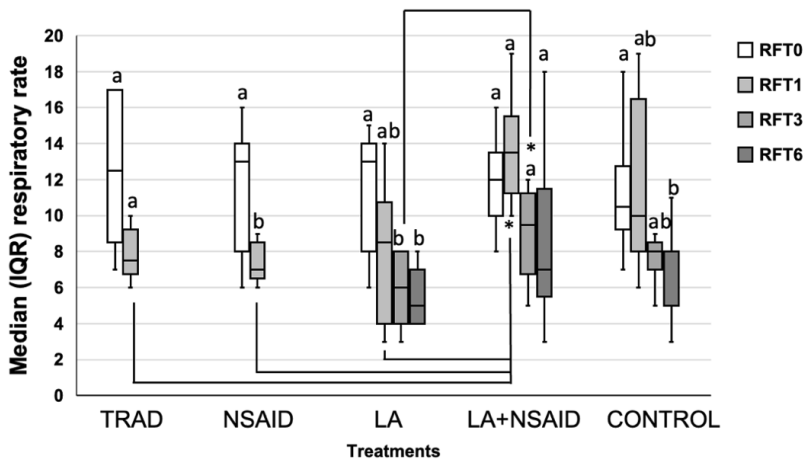


Figure 10 Median (IQR) respiratory frequency (RF breaths/min) during T0 to T6 in clamp-castrated reindeer in five treatment groups: traditional castration (TRAD), castration with meloxicam (NSAID), castration with lidocaine local anesthesia (LA), castration with lidocaine and meloxicam (LA+NSAID) and holding control (CONTROL). Differing letters above columns indicate significant differences ($p < 0.05$) **within** treatments at a specific measurement point, whereas asterisk (*) and black lines illustrate differences ($p < 0.05$) **within** the measurement points **between** treatments. **Bold** text illustrates the corrected mistake in terms after article II was published

5.5. Effects of castration and pain alleviation on activity and daily rhythms (III)

Overall, the reindeer travelled 7.53 ± 0.75 (mean $\pm$ sem) km during the three observation days. The variation was high, and there were no differences between the NSAID and TRAD reindeer in the mean $\pm$ sem overall distances they travelled daily during the three days (6.60 ± 0.57 km vs 8.60 ± 1.54 km). The reindeer moved individually but within the same geographical area over the three days of measurement.

However, the days differed from each other ($p < 0.001$): reindeer travelled longer distances on the first than on the third day after castration (9.09 ± 0.96 km vs 5.75 ± 0.39 km, $p < 0.001$), while distances travelled on the second day (8.20 ± 1.64 km) did not differ from those for either first or third days. Moreover, we established a significant interaction between treatments and days ($p = 0.01$): TRAD reindeer travelled longer distances than NSAID reindeer during the first day after castration (11671 ± 2250 m vs 7083 ± 608 m, $p < 0.05$) with no difference observed on the second and third days (10.19 ± 3.87 km vs 6.59 ± 0.85 km and 5.35 ± 0.39 km vs 6.17 ± 0.70 m, respectively). In addition, distances travelled by NSAID reindeer did not differ between days, but the activity of TRAD reindeer decreased over three days (Figure 11).

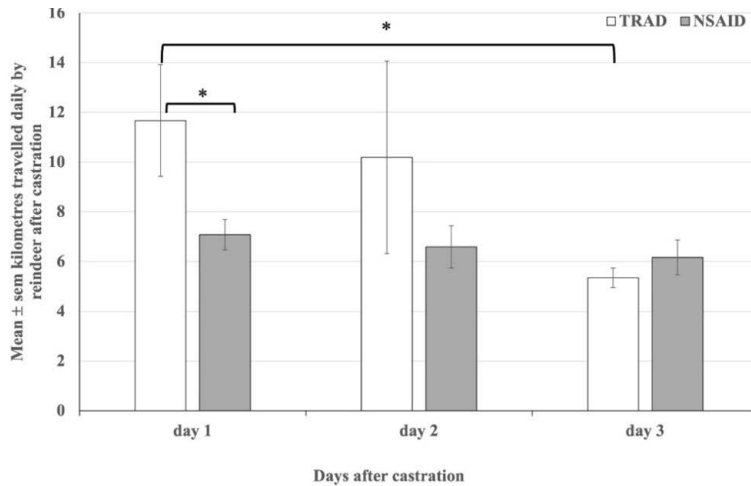


Figure 11 The overall mean ± sem kilometres the newly clamp castrated reindeer travelled in the wilderness. The whiskers and asterisks mark statistically significant differences. NSAID (n = 8) were injected 0.5 mg/kg meloxicam subcutaneously and TRAD (n = 8) was castrated without analgesia

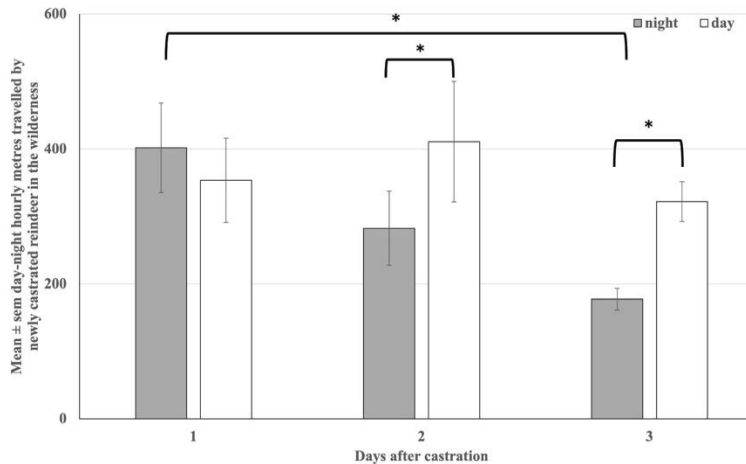


Figure 12 Mean ± sem day-night hourly metres the 16 newly clamp castrated reindeer travelled in the wilderness for 3 days. Statistically significant differences are marked with whiskers and asterisks.

Similarly, the overall hourly distance the reindeer travelled was 272.12 ± 20.72 m during the 3 days, which did not differ statistically between TRAD and NSAID reindeer (302.31 ± 39.91 km vs 244.94 ± 18.61 m, respectively). However, we found differences between the days and hours; overall, reindeer tended to travel longer hourly distances during the first than second day (345.78 ± 31.38 m vs $276.39 \pm$

39.44, $p = 0.05$) and travelled shorter hourly distances on the third (210.85 ± 13.51 m) than on the first day ($p < 0.001$).

The total hourly daytime and night-time distances travelled by reindeer differed ($p < 0.005$); the reindeer travelled shorter distances during night-time than during daytime (271.89 ± 24.88 m vs 360.26 ± 46.06 m, $p = 0.02$). We observed no overall differences between the treatments, but there was an interaction between days and the day-night phases: the total distances travelled by day and by night did not differ on the first day, but longer daytime distances were travelled on days 2 and 3 than during respective nights. The travelled daytime distances remained steady, but the night-time distances decreased (Figure 12). The differences between days and hours were respective to Figure 11 (data not shown).

Overall, the reindeer travelled the longest hourly distances during daytime hours (06:00–15:00). Moreover, there were significant interactions between treatments, days, and hours ($p < 0.001$) showing that the TRAD group was more restless (Figure 13). During the start of the first day, after the restless initial night, the NSAID group had a higher activity peak than TRAD group 08:00–11:00 in the morning. Otherwise, TRAD reindeer travelled longer hourly distances, especially between 15:00–16:00 in the afternoon. However, the variation was high in TRAD. The reindeer generally travelled mostly during daytime hours (07:00–17:00), with several (5–9) activity peaks across the 24-hour day.

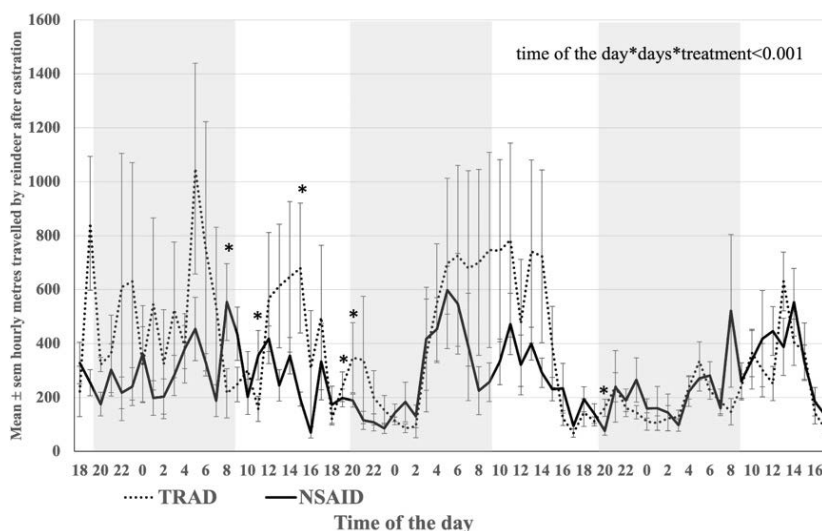


Figure 13 The overall mean $\pm$ sem hourly metres the newly clamp castrated reindeer travelled in the wilderness for 3 days. NSAID ($n = 8$) was injected 0.5 mg/kg meloxicam subcutaneously and TRAD ($n = 8$) was castrated without analgesia. The grey shading marks the night-time. Statistically significant differences are marked with asterisks.

5.5 Mortality and gastric ulcers

After study II, three reindeer were found dead from the trial enclosure. The first two were found dead 14 days after the trial day and autopsied in the Finnish Food Authority Animal Health Diagnostic Unit in Oulu; the cause of death was apathy and languishing, possibly stress related. Both dead reindeer were from the LA treatment group. The third individual was found dead 19 days after the trial and autopsied by two experienced veterinarians in the field. This reindeer was from the NSAID treatment group. A perforating gastric ulcer was discovered, presumably the cause of death.

After transportation of the castrated reindeer to the abattoir in Rovaniemi, gastric ulcers were found post-mortem from ten more reindeer: 2 TRAD, 3 NSAID, 3 LA, 2 LA+NSAID. From study III, performed in Naltio wilderness, 14/16 reindeer were found alive and in good condition the year after the trial. One reindeer from NSAID and one reindeer from TRAD were lost after the 3-week follow-up period.

6 Discussion

This thesis focuses on pain behaviours and pain alleviation in clamp castrated reindeer. Pain, its recognition and alleviation, is an important welfare issue. This thesis shows that oral meloxicam is a promising option for castration pain relief in the field – a single dose of meloxicam (0.5 mg/kg IV or orally) has the potential to maintain the therapeutic concentration determined in other species for up to 2-3 days in reindeer plasma. Reindeer express clamp castration pain by contracting their abdominal muscles and closing their nostrils (i.e. holding their breath), and showing locomotory patterns that might indicate pain-induced change in their activity rhythm in the first days following the procedure. Contrary to the hypothesis, local anesthesia (LA), with a 5-minute effect time, failed to reduce the pain expression behaviours, while the longer handling time waiting for the LA effect caused presumed problems with capture and restraint stress, that is, signs of intimidating hyperthermia. As expected, perioperative NSAID meloxicam does not ease the immediate pain during clamp castration, but it is beneficial for castrated reindeer, changing postoperative activity patterns in a way that suggests pain alleviation during the first 2–3 days following clamp castration.

6.1 This study fills current gaps in knowledge

6.1.1 Wintertime pharmacokinetics of non-steroidal anti-inflammatory drug meloxicam in reindeer after intravenous and oral dosing (I)

The initial specific aim was to investigate meloxicam pharmacokinetics in reindeer in study I, and this was the first ever study to analyse the pharmacokinetics of any NSAID in reindeer. We showed that plasma meloxicam concentrations measured after both IV and oral administration (oral $C_{\max}$ 1.82 (1.17–2.78) µg/ml) were well within the therapeutic concentration of meloxicam as determined in other species (horses 0.13–0.2 µg/ml: Toutain and Cester, 2004; dogs 0.82 µg/ml; Montoya et al., 2004; humans 0.57–0.93 µg/ml; Türck et al., 1996). The therapeutic

concentration in reindeer is yet unknown, but our data suggest that a single IV or oral dose of meloxicam (0.5 mg/kg) may potentially maintain the assumed therapeutic concentration in reindeer from one hour (IV) to three hours (oral) post administration, up to approximately 2–3 days. Our results from study III on the activity patterns of meloxicam treated reindeer after clamp castration support this assumption.

Although study I results on the 49% bioavailability of oral meloxicam are based on three animals only, with complete data from both treatments, it suggests that the oral dosage used in the study (0.5 mg/kg) may need to be doubled for reindeer. The oral dosage was chosen with caution because the main side effect of meloxicam is ulcerogenic activity in the gastrointestinal tract (EMA, 2010) and because reindeer are prone to develop gastric ulcers resulting from stress of handling and herding (Rehlinger, 1990, Wiklund et al., 1996 a,b). Nonetheless, studies in cattle show that meloxicam at 0.5–1.5 mg/kg for 5 days has minimal side effects (EMA, 2010). Therefore, increasing the dosage in reindeer could be considered. This idea is also supported by Türk et al. (1996), who showed that the meloxicam pharmacokinetics in humans is dose-linear. However, maximum plasma concentration (C_{max} median (range) 1.82 (1.17-2.78) $\mu\text{g/ml}$) was clearly higher in reindeer treated with the same oral dosage (0.5 mg/kg) as goats (0.736 ± 0.184 $\mu\text{g/ml}$, Ingvast-Larsson et al., 2011), and at the same level compared with other ruminants treated with a double dosage of 1.0 mg/kg in sheep (1.72 (1.45 -1.93) $\mu\text{g/ml}$, Stock et al., 2013), bovine calves (1.9 (1.3-2.1) $\mu\text{g/ml}$, Glynn et al., 2013), and llama (1.368 (0.826-1.776) $\mu\text{g/ml}$, Kreuder et al., 2012). The present study also demonstrates that $t_{1/2}$ is longer and CL slower in reindeer than in sheep or goats (Shukla et al., 2007; Ingvast-Larsson et al., 2011; Stock et al., 2013). Thus, without toxicity and efficacy studies, the optimal oral dosage in reindeer remains only speculative.

In ruminants, the rumen flora play a crucial role in metabolizing and deactivating contaminants, while binding to digesta slows down absorption (Lees, 2018). Seasonal shifts affect the metabolism of reindeer; during winter, when freely grazing, they adjust to the harsh arctic conditions and limited food availability, significantly reducing their metabolism by over 50 %, leading to a decrease in the activity of rumen and its microbial flora (Mesteig et al., 2000; Åhman & White, 2019; Arnold, 2020). Consequently, drug absorption and metabolism may vary significantly between animals captured from natural grazing grounds in winter compared to those fed in winter enclosures, or during summer. The maximum plasma concentration after oral administration was reached considerably faster in reindeer (t_{max} approximately 6 hrs) than in other ruminant species, such as ruminant bovine calves (11.64 hrs, Coetzee et al., 2009; 17.3 hrs, Mosher et al., 2012; 24.0 hrs, Melendez et al., 2019), pre-ruminant calves (10 hrs, Jokela et al., 2023; 14.3-17.0 hrs, Mosher et al., 2012), goats (15 hrs, Ingvast-Larsson et al., 2011),

llamas (21.4 hrs, Kreuder et al., 2012) and sheep (19 hrs, Stock et al., 2013). The type of pharmaceutical product used may influence the absorption speed. According to the manufacturer, the oral suspension utilized in this study reaches its peak concentration (t_{max}) at 4.5 hours in dogs and at 2–3 hours in horses. We did not collect blood sample for drug analysis between 3 and 6 hours or between 6 and 12 hours post medication so that the time to reach peak concentration and the duration of peak concentration are unknown. The intervals between blood collection were long to minimise the number of samples, i. e. to avoid excessive sampling stress. Therefore, we may speculate whether – from a pharmacokinetic perspective - reindeer during winter could be classified more similar to monogastric animals rather than typical ruminants, but more research is needed to confirm this assumption.

Notably, metabolism of meloxicam may be different in reindeer in summertime. A comparable summer study is, however, not simple to perform for several reasons. Animals would be of a different age than in this winter study (reindeer always calve in May-June), acquiring the free-grazing animals for the study would be challenging, and if acquired, natural feeding would be very difficult to organize in enclosures - reindeer eat numerous different plant species in summertime. Moreover, growing velvet antlers are very vulnerable to handling.

There were some limitations in study I. Our aim was to inspect the wintertime pharmacokinetics of meloxicam in reindeer as the nature of the livelihood creates a larger demand for medication mainly during the winter period, when the free grazing animals are gathered from the wilderness. Reindeer are adapted to the arctic climate with their thick, dense winter hair, which makes venous access difficult in a freezing field setting, with animals insufficiently accustomed to human handling. The study faced additional hurdles from severe winter weather and dealing with less tame animals, resulting in some data loss. Furthermore, the level of plasma protein binding of meloxicam is unknown in reindeer. Protein binding is an important factor in the variance of effective plasma concentrations of NSAIDs (Galbraith and McKellar, 1996). While NSAIDs typically bind strongly to plasma proteins, leading usually to a moderate distribution volume (Türck et al., 1996; Busch et al., 1998), inflammation facilitates the leakage of plasma proteins with bound NSAIDs into damaged tissue (Lees et al., 2004), aiding in targeting the injury site. According to EMEA (2010), meloxicam is at least 96.5 % bound to plasma proteins in cattle. However, NSAID binding to plasma proteins varies greatly among species and cannot be extrapolated from one species to another (Galbraith and McKellar, 1996). Hence, the lack of knowledge regarding protein binding is an important confounding factor when approximating the therapeutic plasma concentration in reindeer.

Although oral administration of meloxicam seems promising for reindeer in field conditions, further experimental research is required on the optimal dosage

and efficacy of meloxicam, evaluating adequacy of analgesia after various dosing routes and possible seasonal differences in pharmacokinetics, and exploring protein-binding, tolerance and toxicity.

6.1.2 The effects of castration on reindeer behaviour, rectal temperature and respiratory rate during the clamp castration procedure, and how these are affected by handling, local anesthesia and a non-steroidal anti-inflammatory drug (II)

As hypothesized, reindeer show behaviours indicative of pain during clamp castration. Abdominal muscle contractions and nostril closures were clearly connected with the moment of crushing the tissue with the clamps. These behaviours were scored neither prior castration nor in the CONTROL group. Clamp castration is painful (Robertson et al., 1994; Fisher et al., 1996; Bretschneider, 2005; Stafford and Mellor, 2005), and a prey animal like reindeer shows subtle pain behaviours, just holding breath with tension in abdominal muscles. The number of head and neck movements, kicking, panting, or defecating were not affected by castration or given medication. Therefore, these behaviours can be interpreted as general struggling behaviours against manual immobilization, as described in Molony and Kent (2011). The role of leg flexions and straightenings as a sign of pain remains unclear. There was an increase in number and duration of leg flexions in long treatments during castration versus CONTROL or prior castration, but this behaviour was also scored when not connected with any assumable cause of pain.

Eye and ear movements and vocalization recognized anecdotally in the field practice were dismissed from the ethogram due to poor visibility (sleet, snow and polar night). This creates a need for future studies in reindeer pain scale and pain face. Visibility was at times generally poor during the long experiment day. Notably, immobilization and holding may also confound the results of scoring the limb movements and experimenting in the enclosure with rather young animals may not completely correlate with field conditions in round-up with adult males, the latter possibly incur more distinct tissue damage (Pieler et al., 2013) and an increased stress response (King et al., 1991; Ting et al., 2003).

Clamp castration is a quick procedure – median procedure time in our research setting was 24.6 seconds for traditional castration. As we hypothesized, prolonged holding leads to a marked rise in rectal temperatures. All the LA+NSAID group were measured higher than 39.5 °C, which is a threshold for selective brain cooling in reindeer (Blix et al., 2011). Eleven percent of the study animals scored higher than 40.0 °C and were at the edge of hyperthermia, for which the clinical point is ≥ 2 °C higher than normal (Arnemo et al., 2014).

Unlike we expected LA with a 5-minutes waiting time failed to remove the pain behaviours. Insufficient anesthetic effect during Burdizzo castration is in line with research performed in other ruminants (Fisher et al., 1996; Stafford et al., 2002; Ting et al., 2003; Mellema et al., 2006; Melches et al., 2007; Thüer et al., 2007; Boesch et al., 2008; Durand et al., 2019), but may also be due to the relatively short period between lidocaine injection and castration. An extended period might improve the anesthetic effect (Thüer et al., 2007). Coetzee (2011) indicated that the waiting time for LA used in various calve castration trials was 5–20 minutes before castration with Burdizzo clamps, even though the onset for the chosen lidocaine for local infiltration is quite rapid, 2–5 minutes (Coetzee, 2013; Weinberg et al., 2015). However, already with the 5-minute waiting time used in study II, the increased handling duration was strongly associated with elevated rectal temperatures, also with the non-castrated control animals, indicating alarming capture and restraint stress.

Moreover, the area affected by the injected lidocaine may have been too restricted in our study despite the long experience of the veterinarian performing the treatment. It is noteworthy that this study was not performed under hectic and challenging field conditions of a reindeer round-up with aggressively fighting bulls in rut. Still, in the ambient conditions (e.g. polar night, freezing minus degrees, sleet, snow, rain), the reliability of injections is affected even with most experienced professional injecting the lidocaine. In this study we used 1.5” needles, long enough to reach the site, and a combination of nerve blockage and infiltration. Absorption of lignocaine (lidocaine) is dependent on the total dose administered (Weinberg et al., 2015) and the dosage we used was calculated according to the safety level of the drug; maximum of 4–5 mg/kg (Caulkett and High, 2004; Coetzee, 2013). Our study animals were young males weighing approximately 65–80 kgs and therefore we ended up giving 10 ml (Lidor 20 mg/ml inject., VetViva Richter GmbH, Austria).

Finally, it is also possible that the behavioural variables observed in our study were not sensitive enough to measure how reindeer experience the strength of pain caused by the castration clamps. Unlike this ethogram, most ethograms referred to in this study (e.g. Robertson et al., 1994; Molony et al., 1995; Molony et al., 2002; Melches et al., 2007; Thüer et al., 2007; Medeiros do Nascimento et al., 2023) were mainly made in post castration pain studies. Thus, even though the mean and total duration of bouts were included in our ethogram, our bouts do not necessarily reveal the variation in the strength of individual pain experiences.

Only when analysing the respiratory frequencies did we see some effects of local anesthesia that may illustrate partial pain relief: during castration TRAD had the highest respiratory rate, differing from LA. All treatments affected the respiratory rate, they were higher than the reference values of 23–36 (lying vs standing) breaths/min (White and Yousef, 1978), but mental and physical stress have also been shown to increase respiration frequency, even up to > 250 breaths/min under

experimental conditions (Blix et al., 2011). It is noteworthy that respiration rate during castration is confounded by nostril closure and abdominal muscle contraction, which means that an individual holds its breath, and therefore these behaviours may affect the respiratory rate. Removing these respiratory pauses from analyses is not feasible due to the shortness of time that the clamps crush the tissue, only 6–7 seconds, so that removing the pause would remove the whole duration of castration.

However, failure of LA to alleviate acute pain behaviours during castration does not rule out the presumed benefit of both lidocaine and perioperative meloxicam, especially if administered 20–30 minutes prior to the procedure. Catching an animal twice represents an additional major stress factor, but nonetheless is possible, especially with tamed tourist reindeer or reindeer otherwise more used to handling and human presence, in other than hectic great round-up situations. Moreover, LA may still be beneficial for alleviating pain during the first hours after the procedure, as demonstrated with lambs (Mellema et al., 2006). Multimodal analgesia, combining NSAID with LA, is expected to be more effective (Stafford and Mellor, 2005; Coetzee, 2011; Durand et al., 2019). Thus, more research is needed on the use of LA, also combined with NSAID, for example on standing reindeer in chute-like constructions, and during the first painful hours post castration. An effective option could be the use of epidural anesthesia, but it may also be problematic in winter if it decreases a reindeer's movement ability for too long a time, and administration is also complicated in the field during the cold season. The longer-term benefits of lidocaine and NSAIDs are yet to be demonstrated in reindeer.

These study findings on increased rectal temperatures during handling indicate that the seasonal timing and treatment method of reindeer castration should be carefully considered, to avoid any extra stress. Reindeer have extremely thick and insulated winter hair, and they are therefore susceptible to stress-induced increase in body temperature (Lian et al., 2019), which was also indicative in this study. Heat stress in reindeer is strongly related to capture method, with additive effects from physiological and mental stressors, like fear, metabolic heat from muscular exertion, anesthetic drugs, or high ambient temperature (Lian et al., 2019). The normal body temperature for resting reindeer is 38.6 ± 0.2 °C (Blix et al., 2011), and rectal temperature may be lower than the core body temperature (Ozeki et al., 2014).

We chose reaching 39.5 °C specifically as a response variable. As the body T seemed to rise very fast with handling, it would have been misleading if only increase in T would have been considered - the basic level may be higher than normal already because of the stressful situation. Stress-induced hyperthermia has been described in multiple wild species (Arnemo et al., 2014). DelGiudice et al. (2001) studied white-tailed deer (*Odocoileus virginianus*) and classified

temperature as “high” when $> 39.4^{\circ}\text{C}$, whereas Roug et al. (2022) studied mule deer (*Odocoileus hemionus*) and actively cooled down animals with rectal body T $> 39.5^{\circ}\text{C}$. Moreover, Blix et al. (2011) showed that signs of heat stress in reindeer start at a brain T of 39.0°C even though the threshold for selective brain cooling during exercise seems to be slightly elevated at 39.5°C .

The longer handling time due to a 5-minute waiting in the long treatment groups caused a significant rise in temperature, especially in reindeer that were administered both LA and NSAID. However, this same effect was not seen in reindeer administered only LA, and reindeer did not react behaviorally to LA administration. The irritation caused by the subcutaneous meloxicam injection (EMEA, 2010; Guo et al., 2021) may possibly have added to the stress caused by restriction. In the short treatment with NSAID, this irritating effect may have been shrouded by other simultaneous issues. Even though the initial rectal temperatures did not differ between treatments, the LA+NSAID group had numerically slightly higher temperatures. Therefore, we cannot rule out that it might have affected the results. As we showed in study I, oral meloxicam is a promising option when treating reindeer, hence, avoiding the assumed irritation caused by subcutaneous meloxicam injection is possible.

Capturing and handling reindeer in general has risks: mortality in our second study was 5.5%, and 20.0% of the study animals were diagnosed with gastric ulcers post-mortem, regardless of the treatment group. This is in line with previous research results (Rehbinder, 1982, 1990; Wiklund et al., 1996 a,b). Additional research is needed with more sensitive measures and for various pain alleviation alternatives in reindeer. We only measured the signs of pain during castration itself. It is at least as important to define signs of post-procedure pain during the first few postoperative hours and days and find some effective means of alleviating it. Until effective treatment can be initiated, in cases of acute distress, the immediate release of the reindeer should be targeted (Lian et al., 2018) until we have suitable means to alleviate acute pain effectively.

6.1.3 The effects of clamp castration and NSAID on activity during three days post procedure (III)

According to our hypothesis, untreated pain affects the activity patterns of reindeer. In study III we showed for the first time that untreated pain is likely to increase the movement of castrated reindeer in the wild. The subcutaneously administered NSAID meloxicam reduces the activity of castrated reindeer in the first two days after castration compared with reindeer castrated traditionally without analgesia. We found that clamp castrated TRAD reindeer were particularly active during the two days after castration, and their activity decreased to the same level with NSAID

reindeer on the third day after castration. However, the NSAID group overall maintained a more consistent level of movement over the first three days. This is well in line with the presumed duration of the therapeutic level of meloxicam estimated in study I and the pain behaviours and tissue swelling after clamp castration shown in other species, the two first post-operative days being the most painful (King et al., 1991; Robertson et al., 1994; Molony et al., 1995).

The overall day-night activity difference disappeared during the first day after round-up and clamp castration but returned from the second day onwards. Immediately after the round-up and castration, reindeer from both treatments were restless during the night following castration. The loss of day-night rhythm can be attributed to several factors, including the stress of gathering and round-up, short-term irritation caused by ivermectin injection reported by the manufacturer (Virbac, 1999), and the potential tissue irritation caused by administration of subcutaneous meloxicam (EMA, 2010), as also suspected in study II. Following the initial night, the reindeer generally resumed a clear diurnal activity pattern, consistent with previous observations (Eriksson et al., 1981).

Notably, because our study was a pilot experiment, we lacked a non-castrated control group. Furthermore, as the rutting season was ongoing, equipping fighting bulls with GPS collars would have raised concerns about animal welfare. Our sample size was limited, especially due to the real-life challenges of collecting the study animals. Thus, our results should be interpreted with caution as increased activity after a painful procedure could either be a positive or a negative sign (Chapman et al., 1985). However, reindeer in general are vigilant and flight-reactive (Baskin and Hjalten, 2001; Reimers et al., 2011; Nieminen, 2013), and thus increased activity in the wild because of pain is not unexpected but in line with other studies on pain and activity (Molony et al., 1993). Additionally, our findings for the NSAID group are supported by comparisons with GPS data from reindeer monitored by the Natural Resources Institute Finland (LUKE), collected in the same area and season, although from female reindeer. The daily 4 km distances travelled by the LUKE group (Kumpula et al., 2015) were more aligned with those of our NSAID-treated reindeer. Our study reindeer mainly moved separately. Social behaviours may play less of a role during the autumn rut with males, even though they tend to form separate groups during summer (Holand et al., 2022).

We faced some challenges in our data collection, dealing with a semi-domesticated species whose individuals are prone to ridding themselves of any foreign objects attached to them, and with animals grazing in the vast wilderness close to the border of Russia. Ultracom GPS tracker sends the location to the server via mobile network, and there are poor coverage spots in the area, the more the further towards east from the Naltio round-up enclosure the reindeer grazed. Moreover, some of the data gaps are probably caused by natural obstacles, i.e. lying

reindeer's neck/torso or natural formations blocking the signal between the GPS sensor placed in a collar, and the satellites.

The accuracy of the GPS collar signal was notable: the three fallen collars were found from the snowy wilderness according to the last signal that was sent. Several blind spots were apparent when collecting GPS signals and reindeer locations, and these blind spots increased the further our study animals grazed from the round-up enclosure. We followed the reindeer with GPS for three weeks but excluded data from day 4 onwards from the analysis. Despite the marked variation and missing data, the analysis was sensitive and sufficient for answering our research question, showing that reindeer appear to benefit from NSAIDs administered at the time of clamp castration, making GPS collars promising devices for future pain studies in free-ranging reindeer.

6.2 Current best practice and future concerns

The thesis fills the current gaps in knowledge: the first ever study on the pharmacokinetics of an NSAID, i.e. meloxicam, was successfully accomplished and the first ever ethogram for reindeer pain behaviour was created as a tool to show that clamp castration is painful. We showed that perioperative LA and NSAID are not optimal choices for alleviating pain during castration, and that prolonged handling creates problems with a rapidly increasing rectal temperature. However, we also showed that untreated pain affects the activity patterns of reindeer post clamp castration.

Animal welfare legislation has been revised in Finland (693/2023) and pain alleviation for painful procedures is mandatory from January 2024 onward. There has been a gap in knowledge on the practical means of medicating reindeer, targeting castration pain alleviation, because these animals are kept on a free-range system, they are less used to human presence and are easily excitable, field anesthesia is problematic and capture and prolonged restraint may cause problems, even mortality. Therefore, there is a conflict between the animal welfare demands and the lack of evidence-based medicine for reindeer, given that some good means may, in the worst-case scenario, do more harm than good for this species.

Pain alleviation in reindeer is challenging. Their wintertime metabolism can slow down by more than 50% (Mesteig et al., 2000; Pösö, 2005; Arnold, 2020), which may have a great impact on the pharmacokinetics of different drugs (Laitinen et al., 1980a,b, 1982a,b; Juvonen et al., 1984). Sedation and/or narcosis is dangerous in wild ruminants, causing severe side effects (Ryeng et. al., 2001; Caulkett and High, 2004), especially during harsh winter conditions and hectic round-up situations. Moreover, many of the sedative drugs available, especially antagonists, are not accepted for animals intended for food production. Local anesthesia adds to handling time or handling occasions and this species is, in

general, prone to handling stress and problems following handling (e.g. hyperthermia, gastric ulcers, capture myopathy) (Rehbinder, 1982, 1990; Wiklund et al., 1996a,b; Paterson, 2014). NSAIDs, on the other hand, are not sufficient to treat acute pain because of the tissue damage caused by clamp castration (Stafford and Mellor, 2005; Coetzee, 2011), and the evidence base has been missing on their use with reindeer. These drugs are used off-label according to the cascade rules of the EU (2019/6) directive. Research on other ruminants shows that they should be administered at least 20 minutes before the painful procedure (Coetzee, 2011), which also adds to handling stress with repeated handling occasions. Subcutaneous injection may be irritating (EMEA, 2010).

According to current best knowledge, if castration is considered necessary, the justifiable way for a reindeer, less used to human presence and handling, is a quick, careful procedure combined with NSAID for postoperative analgesia, helping the bull to recover during the following days. Hypothetically, if the reindeer is already tamer and more used to human presence, administering LA 20-30 minutes before the operation, while the reindeer is standing, along with simultaneously administered NSAID either subcutaneously or orally (oral ideally 3-6 hours prior to the procedure), could potentially enhance the welfare of the reindeer.

Why not avoid castration totally, if no effective means of acute pain alleviation exists? There are no straightforward solutions. Minimizing or preventing pain involves reevaluating the methods used for procedures and questioning their necessity. Ultimately, achieving a reduction in animal pain calls for a combination of scientific innovation and a shift in cultural values (Weary et al., 2006). Such innovation could be, for example, the development processing of chemical castration, already used for pigs (Zamaratskaia et al., 2008, EMEA, 2009). However, the reindeer must be caught also for immunocastration injections and implants, and even sedated (Marliani et al., 2023), which makes their use questionable.

Slaughtering intact males could be feasible in certain circumstances, either before or after the rut and rehabilitation, especially in a diverse reindeer herding area with 54 cooperatives exhibiting varying geography, land area, practices, and realities. Our questionnaire, yet to be published, exploring various herder attitudes to reindeer castration and pain, reveals a near-even split among herders regarding the question of slaughtering males intact, with approximately 52% in favor and 48% against.

Traditional bull slaughter before the rutting season is well established in neighbouring countries (Holand et al., 2022). In Finland, the primary focus of economic meat production centers around slaughtering calves, with the optimal slaughter age for reindeer bulls identified as five years (Pekkarinen, 2018). Gathering reindeer herds before the rut, when calves are still too small, would impose significant additional stress on the herds. Additionally, keeping bulls in

enclosures during the rut is impractical due to their aggressive behaviour and propensity to fight. Releasing and recapturing individuals later is both time-consuming and labor-intensive. Slaughtering bulls after rehabilitation at farmyard enclosures also contributes to increased feeding costs. However, slaughter of intact males is still performed by some herders in Finland.

Future research is needed on the safe and effective means of pain alleviation for inherently painful procedures, and on the means for entirely avoiding such procedures. Motivating the herders by raising awareness of animal pain and stress, and their role in not only wellbeing but survival in general, plays a crucial role in successful practices in the field.

Conclusions

Pain alleviation in reindeer clamp castration is an animal welfare issue. Reindeer are prey animals and show subtle castration pain behaviours, but pain is still evident. However, some means of analgesia may cause greater problems to reindeer caused by capture and prolonged restraint. NSAID meloxicam is beneficial for reindeer post castration and can be administered orally, which makes it a promising and producer-friendly option in the field. To date, the therapeutic concentration for reindeer remains unknown.

Major findings reported in this thesis:

1. Reindeer express clamp castration pain by contracting their abdominal muscles and closing their nostrils (i.e. holding breath) and showing locomotory patterns that might indicate pain-induced change in their activity rhythm in the first days following the procedure.
2. LA failed to reduce the acute pain expression behaviours, while the longer handling time waiting for the LA effect caused problems with capture and restraint stress (hyperthermia).
3. Perioperative NSAID (meloxicam) does not help the acute pain during castration, but it is beneficial for the reindeer, changing postoperative activity patterns in a way that suggests pain alleviation during the first 2-3 days following clamp castration. Orally administered meloxicam is a promising option.

Further research is needed with more subtle measures of pain related to castration and of the effective means of pain alleviation, or scientific innovations or practices of avoiding painful procedures completely. Future experiments are also needed on the efficacy of meloxicam, evaluating the optimal dosage and adequacy of analgesia after various dosing routes, and to explore protein-binding, tolerance and toxicity.

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RESEARCH PAPER

Wintertime pharmacokinetics of intravenously and orally administered meloxicam in semi-domesticated reindeer (*Rangifer tarandus tarandus*)

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Abstract

Objective To investigate the pharmacokinetics of orally and intravenously (IV) administered meloxicam in semi-domesticated reindeer (*Rangifer tarandus tarandus*).

Study design A crossover design with an 11 day washout period.

Animals A total of eight young male reindeer, aged 1.5–2.5 years and weighing 74.3 ± 6.3 kg, mean $\pm$ standard deviation.

Methods The reindeer were administered meloxicam (0.5 mg kg^{-1} IV or orally). Blood samples were repeatedly collected from the jugular vein for up to 72 hours post administration. Plasma samples were analysed for meloxicam concentrations with ultraperformance liquid chromatography combined with triple quadrupole mass spectrometry. Noncompartmental analysis for determination of pharmacokinetic variables was performed.

Results The pharmacokinetic values, median (range), were determined. Elimination half-life ($t_{1/2}$) with the IV route ($n = 4$) was 15.2 (13.2–16.8) hours, the volume of distribution at steady state was 133 (113–151) mL kg^{-1} and clearance was 3.98 (2.63–5.29) mL hour^{-1} kg^{-1} . After oral administration ($n = 7$), the peak plasma concentration (C_{max}) was detected at 6 hours, $t_{1/2}$ was 19.3 (16.7–20.5) hours, C_{max} 1.82 (1.17–2.78) $\mu\text{g mL}^{-1}$ and bioavailability ($n = 3$) 49 (46–73)%. No evident adverse effects were detected after either administration route.

Conclusions and clinical relevance A single dose of meloxicam (0.5 mg kg^{-1} IV or orally) has the potential to maintain the therapeutic concentration determined in other species for up to 3 days in reindeer plasma.

Keywords animal welfare, cervid, meloxicam, NSAID, reindeer, ruminant.

Introduction

Reindeer herding remains a vital livelihood, dating back centuries, and is one of the oldest livelihoods in Fennoscandia. Based on a legislated right (Reindeer Herding Act 848/1990) to graze freely on the landscape covering one-third of Finland, reindeer herding differs remarkably from other forms of animal husbandry, where production animals are mainly kept in shelters and fenced areas. The freely grazing herds are transferred from the wilderness to fenced enclosures twice a year, that is, in the summer for calf marking and in the fall/winter for the round-ups, where the reindeer are counted, earmarked, treated with antiparasitic drugs and separated for slaughter. Those males not required for breeding are castrated, mostly without pain alleviation. A share of the reindeer are kept in separate winter enclosures or unfenced areas for supplemental feeding during the coldest winter months, while the majority of the animals graze freely in the wilderness all year round.

Veterinarians routinely use off-label analgesia on reindeer to treat various inflammatory conditions and injuries. Nevertheless, pain management in reindeer is challenging. Currently, no analgesic drugs have been approved for the European market for this semi-domesticated species. However,

according to cascade rules in Europe, the use of drugs approved for other production animal species is accepted (Directive 2004/28/EC). The dosages in reindeer practice are extrapolated from other ruminant species. The medication of these semi-domesticated, freely grazing animals is confounded by the slowing of metabolism by even more than 50% during the harsh winter months (Mesteig et al. 2000; Pösö 2005; Arnold 2020), affecting drug absorption and metabolism. Furthermore, reindeer are susceptible to handling stress (Rehbinder et al. 1982; Rehbinder 1990; Wiklund 1996; Wiklund et al. 2001; Omsjoe et al. 2009).

Meloxicam is one of the non-steroidal anti-inflammatory drugs (NSAIDs) used for reindeer, for alleviating pain and various inflammatory processes (Laaksonen, 2018). However, no studies have been published concerning the pharmacokinetics of NSAIDs in reindeer. Only a few case reports describe the use of meloxicam in small numbers of animals (Pizzi et al. 2011; Gonzalez-Alonso-Alegre et al. 2013; Monticelli et al. 2017). The pharmacokinetic parameters of meloxicam vary significantly between dosing routes and even between ruminant species (Shukla et al. 2007; Coetzee et al. 2009; Ingvast-Larsson et al. 2011; Kreuder et al. 2012; Stock et al. 2013; Karademir et al. 2016; Woodland et al. 2019; Hempstead et al. 2020). Therefore, extrapolating dosages between species is not a good practice. As the need for medication in reindeer arises mainly during the cold season, influenced by the nature of the reindeer herding practices, our objective was to describe the wintertime pharmacokinetics of intravenously (IV) and orally administered meloxicam in reindeer.

Materials and methods

Animals and management practices

The study was approved by the Animal Experimental Board (ESAVI/36846/2019) and Finnish Medicines Agency (Vetkl-nro 04/2019). The study was conducted in February 2020 at the University of Helsinki Reindeer Research site, located in Savukoski, Finland. A total of eight healthy intact male reindeer (six animals born approximately in May–June 2018 and two animals in May–June 2017), aged 1.5–2.5 years and weighing 74.3 ± 6.3 kg, mean $\pm$ standard deviation, were obtained from the Kemin-Sompio reindeer herding cooperative for inclusion in this study. The sample size was determined according to previous published research on other ruminant species. The animals were leased from a single reindeer herder, who signed an informed consent. Freely grazing animals were captured from natural pastures in connection with the round-ups and acclimatized to their new environment for approximately 1.5–2.0 months (depending on each individual animal caught) before the study. During acclimatization, the reindeer were kept together in a fenced forest enclosure covering

approximately 5 hectares (12.36 acres) and fed with commercial feed (Poro 2 Balans; Kinnusen Mylly Ltd, Finland), lichen and dried hay. The animals had sufficient fresh, soft snow to fulfil their water requirements, as described by Soppela et al. (1988).

One week before the experiment, the free movement of the reindeer was restricted with a halter and a 3 m rope, which is a traditional method for taming reindeer for handling. It provided the animals with an approximately 28 m² area for moving. Each animal was individually fed within their area, and additional lichen was used as a reward feed. If needed, their location was changed to keep the snow clean and soft for the reindeer to eat and rest. Food was not withheld before the trials. Reindeer were weighed 3 days prior to the first trial with a simple hanging scale and cargo straps, followed by a clinical examination (visual inspection, auscultation of the heart, lungs and rumen).

Inclusion criteria were normal body condition, no signs of infectious diseases in the 2 weeks before the study, and no special remarks in the clinical examination. A reindeer was excluded if clinical signs of disease developed during the study. The animals were released back to the wilderness after the withdrawal period for meloxicam was complete (28 days according to the cascade rules).

Treatments and blood sampling

A partial crossover design with an 11 day washout period was used. A professional reindeer herder, with an assistant, manually restrained each reindeer for handling. The reindeer was restrained in standing position with the neck bent to one side to allow jugular vein puncture. The hair was not shaved, and IV catheters were not used because the weather conditions ranged from 0 °C to almost –30 °C. Hairless skin renders reindeer vulnerable to frostbite, the blood may have frozen in the catheter and furthermore, reindeer tend to dispose of any foreign materials attached to them.

For the first trial, the reindeer herder selected the tamest four reindeer from the group to facilitate IV administration of meloxicam (0.5 mg kg⁻¹; Metacam, 20 mg mL⁻¹, Boehringer Ingelheim Vetmedica GmbH, Germany). A 3 mL syringe with an 18 gauge needle was used. Meloxicam (0.5 mg kg⁻¹; Metacam, 15 mg mL⁻¹, oral suspension for horses; Boehringer Ingelheim Vetmedica GmbH) was introduced into the oral cavity of the remaining four reindeer with a 3 mL syringe.

Blood samples (4 mL) were collected from the jugular vein using 18 gauge needles and 4 mL vacuum ethylenediaminetetraacetic acid tubes at predetermined times after meloxicam administration: 1, 3, 6, 12, 24, 36, 48 and 72 hours for the IV treatment and 3, 6, 12, 24, 36, 48 and 72 hours for the oral treatment, respectively. The reindeer were kept with a halter and tied rope during sample collection but

released into a 5 hectare enclosure for the 11 day washout period and the withdrawal period.

After the washout period, the animals were captured again the day before the second trial. They were moved from the 5 hectare enclosure to a small corral to be caught by hand and then tied with a rope. A baseline blood sample was taken at the time of the clinical examination, for measurement of meloxicam plasma concentration, to ensure the adequacy of the washout period. An injured pelvic limb was observed in one reindeer which was excluded from the second trial. The second trial was performed according to the crossover design, animals first in the IV treatment now assigned to the oral treatment, and *vice versa*.

Blood samples were stored on snow, and the plasma was separated by centrifugation for 10 minutes at 1509 *g* immediately after blood was collected from all eight reindeer. Afterwards, the plasma was stored at -20°C until analysed.

Meloxicam concentration in plasma

A quantitative analysis of meloxicam was performed with ultraperformance liquid chromatography combined with triple quadrupole mass spectrometry (Waters Acquity UPLC + Waters Xevo TQ-S triple quadrupole MS, Waters Corp., MA, USA) with a reversed-phase column (Waters Acquity HSS T3, 1.8 μm , 2.1×30 mm; Waters Corp.). Plasma samples were prepared for analysis by treating them with a precipitation agent: acetonitrile containing 1% formic acid and 50 ng mL^{-1} tenoxicam as an internal standard (50 μL sample + 150 μL precipitation solution). Samples were mixed for 3 minutes and centrifuged for 20 minutes at 2182 *g*. The obtained supernatant was diluted with an equal volume of 150 mM phosphate-buffered saline, and the sample was submitted to analysis.

The chromatography consisted of a gradient elution of 0.1% formic acid (eluent A) and acetonitrile (eluent B) at a flow rate of 0.500 mL minute^{-1} with a gradient of 5–5–80–98–98–5% eluent B at 0–0.3–1.25–1.3–1.8–2.5 minutes. Injection volume was 4 μL and the temperature of the column oven was 40°C . The analysis was performed with positive ionization. Meloxicam was quantitatively analysed with multiple reaction monitoring mass spectrometry (MRM) transitions of $352 > 115$ and $352 > 184$, with respective collision energies of 19 eV and 15 eV and a retention time of 1.29 minutes. Tenoxicam was quantitatively analysed with an MRM transition of $338 > 121$, with a collision energy of 22 eV and a retention time of 1.02 minutes. MassLynx 4.2 software (Waters Corp.) was used to process the obtained data.

Calibration standards and quality control (QC) samples were prepared by spiking pooled blank reindeer plasma to concentrations ranging from 0.2 ng mL^{-1} to 10,000 ng mL^{-1} for the standards and at 2.5, 25 and 250 ng mL^{-1} for the QC samples. Standards and QCs were treated similarly to actual samples.

The lower limit of quantification (LLOQ) was 0.5 ng mL^{-1} with the coefficient of determination (R^2) > 0.997 , accuracies ranging from 82.8% to 113.7%, and the coefficient of variation (CV%) ranging from 0.5% to 9.6% above the LLOQ. Accuracy and CV at the LLOQ were 82.8% and 13.4%, respectively.

Noncompartmental analysis

Noncompartmental analysis was used to calculate the following pharmacokinetic parameters from the concentration–time–dose data (WinNonlin 5.2; Certara, NJ, USA; Linear Trapezoidal LinearLog Interpolation, uniform weighting): area under curve (AUC), maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), mean residence time (MRT), clearance (CL), and volume of distribution at the steady state (V_{ss}), last two variables for the IV route only. Elimination half-life was calculated with the formula: $t_{1/2} = \ln 2 \times \text{MRT}$ ($\ln 2$ = the natural logarithm of 2).

Results

Plasma concentration is expected to peak immediately after IV injection; however, t_{max} was measured later than the first collected blood sample in three reindeer in the IV treatment. This difference probably resulted from incomplete IV administration, and the data were excluded from analysis (Table 1). Because one reindeer had already been excluded before the second trial, complete series of blood samples were analysed from four animals in the IV treatment. Data were missing (laboratory error) at 6 hours post administration in one reindeer in the oral treatment, and all data from this animal were excluded from analysis (Table 1).

Measured meloxicam concentrations in reindeer plasma over time after a single dosage of 0.5 mg kg^{-1} administered IV or orally are presented in Figure 1. Pharmacokinetic parameters are presented in Table 2. The t_{max} of orally administered meloxicam was 6 hours and plasma mean half-life was 19.3 (16.7–20.5) hours (Table 2). No evident adverse effects were detected after either treatment. Bioavailability (F) calculated for the three reindeer with complete data for both treatments was 49 (46–73)%, median (range).

Discussion

No studies evaluating the pharmacokinetics of NSAIDs in reindeer (*Rangifer tarandus tarandus*) were found in the literature, even though analgesia should be employed for ethical and animal welfare reasons. The results of the present study indicate that approximately half of the orally administered meloxicam reached systemic circulation. In addition to good bioavailability, meloxicam had a long $t_{1/2}$ in both IV and oral treatments, suggesting that the oral route is a feasible option when medicating reindeer with meloxicam.

Table 1 Meloxicam (0.5 mg kg^{-1}) was administered intravenously (IV treatment) and orally (oral treatment) to eight reindeer in a crossover study. Data included in the analysis (✓), data excluded (✗), with cause of exclusion, and bioavailability (F) are indicated for each animal.

Reindeer identification	Oral treatment	IV treatment	F reported
1	✓	✗ (t_{max} delay)	✗
2	✓	✓	✓
4	✓	✗ (injured)	✗
22	✓	✗ (t_{max} delay)	✗
37	✓	✗ (t_{max} delay)	✗
41	✓	✓	✓
47	✓	✓	✓
49	✗ (laboratory error)	✓	✗

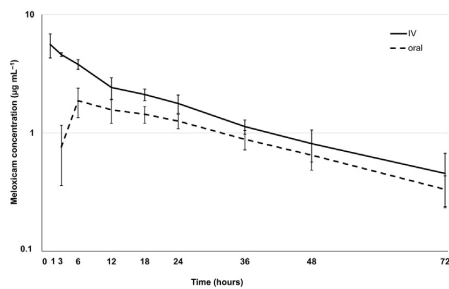


Figure 1 Mean $\pm$ standard deviation plasma meloxicam concentrations (in a half-logarithmic scale) in semi-domesticated reindeer after administration of single doses (0.5 mg kg^{-1}) of oral ($n = 7$) and intravenous ($n = 4$) meloxicam.

Table 2 Median (range) values for pharmacokinetic parameters of meloxicam after administration of a single dose (0.5 mg kg^{-1}) to semi-domesticated reindeer through intravenous (IV) ($n = 4$) and oral ($n = 7$) routes.

Parameters	Oral treatment	IV treatment
$t_{1/2}$ (hours)	19.3 (16.7–20.5)	15.2 (13.7–16.8)
t_{max} (hours)	6 (6–6)	na
C_{max} ($\mu\text{g mL}^{-1}$)	1.82 (1.17–2.78)	na
AUC_{last} ($\text{hour} \cdot \mu\text{g mL}^{-1}$)	62.4 (51.5–88.6)	130.7 (102.9–876.0)
MRT_{last} (hours)	27.8 (24.1–29.6)	22.0 (19.8–24.3)
CL ($\text{mL}^{-1} \text{ hour}^{-1} \text{ kg}^{-1}$)	na	3.98 (2.63–5.29)
V_{ss} (mL kg^{-1})	na	133 (113–151)

AUC_{last} , area under the concentration-time curve from dosing (time 0) to the time of the last measured concentration; CL, clearance; C_{max} , maximum plasma concentration; MRT_{last} , mean residence time; na, not determined; $t_{1/2}$, elimination half-life, t_{max} , time to maximum plasma concentration, V_{ss} , volume of distribution at steady state.

The present study supports previous results that meloxicam absorption is delayed in ruminants after oral dosing (Lees et al. 2004). In the reindeer in the present study, plasma meloxicam concentrations reached peak concentrations 6 hours after oral administration, which would be an undesirable delay for treatment of acute pain. However, blood samples were not collected for drug analysis between 3 and 6 hours or between 6 and 12 hours post medication so that the time to reach peak concentration and the duration of peak concentration are unknown. The intervals between blood collections were long to minimize the number of samples and avoid excessive sampling stress.

Although the 49% bioavailability is based on three animals only with complete data from both treatments, this suggests that the oral dosage used in the study (0.5 mg kg^{-1}) may need to be doubled in reindeer. The oral dosage was chosen with caution because the main side effect of meloxicam is ulcerogenic activity in the gastrointestinal tract (EMA 1999) and because reindeer may develop gastric ulcers resulting from stress of handling and herding (Rehbein 1990). Nonetheless, studies in cattle show that meloxicam at 0.5 – 1.5 mg kg^{-1} for 5 days has minimal side effects (EMA 1999); therefore, increasing the dosage in reindeer could be considered. This idea is also supported by Türk et al. (1996) who showed that the meloxicam pharmacokinetics in humans is dose-linear. However, when compared with goats treated with the same dosage as that used in the present study (Ingvald-Larsson et al. 2011), the C_{max} in reindeer was clearly higher. Furthermore, oral C_{max} in reindeer was at the same concentration as reported after an oral dosage of 1.0 mg kg^{-1} in sheep (Stock et al. 2013), calves (Glynn et al. 2013), and llama (Kreuder et al. 2012). Thus, without toxicity and efficacy studies, the optimal oral dosage in reindeer remains speculative.

The C_{max} after oral administration was reached considerably faster in reindeer than in other ruminant species, such as bovine calves (Coetzee et al. 2009; Melendez et al. 2019), goats (Ingvald-Larsson et al. 2011), llamas (Kreuder et al. 2012) and sheep (Stock et al. 2013). Rumen flora are effective in metabolizing and inactivating contaminants, and binding to digesta delays absorption (Lees 2018). Seasonal changes occur in reindeer metabolism; during the winter, freely grazing reindeer adapt to the Arctic climate and scarce nutrition, lower their metabolism even more than 50% and the rumen and microbial flora enter a resting state (Mesteig et al. 2000; Arnold 2020). However, if additionally fed during the winter months, reindeer continue ruminating (Åhman & White 2018). Consequently, the drug absorption and metabolism may be considerably different when an animal is captured from natural grazing lands in winter, in contrast to an animal fed for weeks or months in an enclosure or during summer. According

to the manufacturer, the oral suspension used in the present study reaches $t_{\max}$ at 4.5 hours in dogs and at 2–3 hours in horses. The results of the present study on $C_{\max}$ and $t_{\max}$ indicate that from a pharmacokinetics perspective reindeer in wintertime could be considered a monogastric animal rather than a ruminant.

In the present study, plasma meloxicam concentrations measured after both IV and oral administration were within the therapeutic concentration of meloxicam as determined in other species. However, reported therapeutic concentrations of meloxicam vary considerably from 0.13–0.2 $\mu\text{g mL}^{-1}$ in horses (Toutain & Cester 2004), 0.82 $\mu\text{g mL}^{-1}$ in dogs (Montoya *et al.* 2004) and 0.57–0.93 $\mu\text{g mL}^{-1}$ in humans (Türck *et al.* 1996). The therapeutic concentration in reindeer is yet unknown. The present study also demonstrates that $t_{1/2}$ is longer and CL slower in reindeer than in sheep or goats (Shukla *et al.* 2007; Ingvast-Larsson *et al.*, 2011; Stock *et al.* 2013). Thus, after the concentration determined therapeutic in a dog (Montoya *et al.* 2004) or a human (Türck *et al.* 1996) was reached in reindeer, it was maintained for at least 24 hours after oral administration and for 36 hours after IV administration. When compared with a horse (Toutain & Cester 2004), our data suggest that a single IV or oral dose of meloxicam (0.5 mg kg^{-1}) may potentially maintain therapeutic concentration in reindeer for approximately 2–3 days.

Regarding AUC, it was more justifiable to report AUC to the time of last measured concentration (AUC_{last}) than AUC extrapolated to infinite time (AUC_{inf}) here. Extrapolating the latter may not be sufficiently accurate because blood sampling was finished at the time (72 hours) when a marked concentration of meloxicam was still detected in the plasma samples. Respectively, MRT_{last} was reported instead of MRT_{inf} , as MRT is greatly influenced by the terminal phase measurements, and its estimate may therefore be unreliable in this study design.

The specific aim in the present study was to inspect the wintertime pharmacokinetics of meloxicam in reindeer, as the nature of the livelihood creates a larger demand for medication mainly during the winter period when the freely grazing animals are gathered from the wilderness. Reindeer are adapted to the Arctic climate with their thick, dense winter hair, which made venous access difficult in the freezing field setting, with animals insufficiently accustomed to human handling. Harsh winter weather conditions and captive wild animals caused extra challenges in the present study, resulting in some results being lost. Furthermore, the level of plasma protein binding of meloxicam is unknown in reindeer. Protein binding is an important factor in the variance of effective plasma concentrations of NSAIDs (Galbraith & McKellar 1996). NSAIDs bind strongly to plasma proteins and, therefore, the volume of distribution is usually moderate (Türck *et al.* 1996; Busch *et al.* 1998), but inflammation facilitates the leakage of plasma

proteins with bound NSAID into tissue damage exudate (Lees *et al.* 2004) assisting NSAIDs to the injury sites. According to EMEA (1999), meloxicam is at least 96.5% bound to plasma proteins in cattle. However, NSAID binding to plasma proteins varies greatly among species and cannot be extrapolated from one species to another (Galbraith & McKellar 1996). Therefore, missing knowledge of protein binding is an important confounding factor when approximating the therapeutic plasma concentration in reindeer.

Conclusions

The present study indicates that meloxicam administered by IV or oral route is a promising option for reindeer in field conditions. However, the therapeutic concentration in reindeer remains unknown. Further experimental research on efficacy of meloxicam is required, evaluating adequacy of analgesia after various dosing routes and to explore protein-binding, tolerance and toxicity.

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Authors' contributions

HN: study design, data acquisition, analyses and interpretation, manuscript preparation, funding acquisition. SL: study design, data acquisition and interpretation, manuscript preparation. MR: study design, data analyses and interpretation, manuscript preparation. LH: study design, data acquisition and interpretation, manuscript preparation, funding acquisition.

Conflict of interest statement

The authors declare no conflict of interest.

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The impact of clamp castration on the behaviour and body temperature of reindeer (*Rangifer tarandus tarandus*) – Effects of local anesthesia and non-steroidal anti-inflammatory drug.

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ABSTRACT

Yearly, 2500–4000 reindeer (*Rangifer tarandus tarandus*) bulls are castrated in Finland, mostly without pain alleviation. No previous research exists concerning pain-related behaviours during castration in reindeer. Our aims were to investigate the effects of Burdizzo clamp castration on reindeer behaviour and rectal temperature during the procedure, and how these are affected by handling, local anaesthesia (LA) with lidocaine, and a non-steroidal anti-inflammatory (NSAID) drug meloxicam. A total of 44 1.5-year-old-reindeer were clamp castrated with either no pain alleviation (TRAD), perioperative meloxicam (NSAID), local lidocaine anaesthesia (LA), or with a combination of LA and NSAID (LA+NSAID), $n = 11$ in each treatment group. The fifth group was a non-castrated control (CONTROL), $n = 11$. We scored the reindeer behaviours, i.e., head, neck, and leg movements, nostril closure, panting, defecation, abdominal muscle contractions, and respiratory rate, from video recordings prior to and during castration and LA injection. We measured rectal temperatures (T) during the experiment and scored the number of reindeer reaching a T of at least 39.5°C. Differences between treatments were analysed with the Kruskal-Wallis and chi square tests and differences between sampling intervals with the Wilcoxon signed rank and Friedman tests. During castration, nostril closures and abdominal muscle contractions occurred more often and for longer duration in the TRAD, NSAID, LA, and LA+NSAID groups than in the CONTROL group ($p < 0.05$ for all) or prior to castration ($p < 0.001$ for all). T increased during the experiment for all treatments. Overall, a total of 25 animals reached T 39.5°C and treatments differed ($p < 0.05$): a larger number reached T 39.5°C in the LA+NSAID group (11/11) than in the TRAD (2/10), NSAID (3/11), LA (4/11), or CONTROL (5/11) groups. The median respiratory rates decreased overall during the experiment for all treatments. During castration, TRAD had the highest median respiratory rate, differing from LA (38(40) vs. 32(20) breaths per min, $n = 10$ and 11, respectively, $p = 0.003$). Reindeer appear to express pain during clamp castration by closing their nostrils and contracting their abdominal muscles. Local lidocaine anaesthesia failed to significantly reduce the pain-related behaviours, at least with the 5-minute effect time used in this study. Moreover, a long handling time increased the rectal temperature of reindeer, especially when combined with other stressors such as injecting NSAIDs.

1. Introduction

Approximately 2500–4000 reindeer bulls are castrated annually in Finland, mostly without pain alleviation (Reindeer Herders' Association statistics). Castration is performed by crushing the spermatic cord with emasculator clamps, and it is justified by better slaughter condition

(Wiklund et al., 2008) when these semi-domesticated, year-round freely grazing animals are gathered from the grazing lands during the years following castration. According to mostly anecdotal information, castrated animals are easier to handle and survive the rutting period better, with less weight loss and fighting injuries. Male reindeer are mostly castrated at around five years of age, when they are no longer needed for

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breeding purposes. Some younger males are removed from breeding use and are castrated purely for meat production purposes. Racing reindeer and tourist reindeer are mostly castrated at three to four years of age to tame them (Laaksonen, 2018).

Based on research performed on other ruminants, clamp castration, like all other castration methods, is considered clearly painful (Robertson, 1994; Fisher, 2001; Bretschneider, 2005; Stafford and Mellor, 2005). Reindeer, like other prey animals, avoid showing signs of pain to predators (Hudson et al., 2008; Tschoner, 2021). However, challenges in recognizing and evaluating masked pain behaviours do not surpass the reasonable assumption that all mammals experience painful procedures in a comparable manner, with similar neural pathways to pain sensation (Hudson et al., 2008; Tschoner, 2021). Bovine calves and lambs express acute castration pain by foot stamping, tail wagging (Robertson, 1994), struggling, kicking (Thüer, 2007; Melches et al., 2007), head and leg movements, urinating or defecating, and vocalization (Meléndez et al., 2018). An increased respiratory rate may be an obvious sign of pain (Kata et al., 2014), but an increase in respiratory frequencies may also reveal mental and physical stress and hyperthermia in reindeer (Lian et al., 2018). No studies exist on how acute castration pain alters reindeer behaviour.

Modern time reindeer are semi-domesticated production animals that graze freely in large herds and are less used to human presence; repeated or prolonged handling must therefore be regarded as unfavourable for the subspecies (Rehinder et al., 1982). These animals are easily excitable, and various measures connected with herding and handling may cause both physiological and emotional distress (Lian et al., 2018). Reindeer are susceptible to capture myopathy, a non-infectious, metabolic disease of wild and domestic animals that is a morbid and mortal outcome of stress caused for example by fear, pain, pursuit, capture, restraint, and transportation (Paterson, 2014; Breed et al., 2019). Captured and restrained reindeer are also prone to a stress-induced increase in body temperature, exhibited as panting when the brain temperature reaches 39.0 °C (Blix et al., 2011). Physical restraint has been shown to cause greater stress compared with chemical restraint (Boesch et al., 2011; Arnemo et al., 2014).

In practice, castration pain can be managed with local anaesthesia (LA), with or without sedation and/or narcosis, and postoperative pain can be treated with peroral or injected nonsteroidal anti-inflammatory drugs (NSAIDs) (Coetzee, 2011). Handling stress is especially relevant in connection with LA administration, as administration and the onset of analgesic activity are separated by a time delay of at least 2–5 min (Coetzee, 2013). If the pain alleviation method increases stress because of prolonged restraining (manual immobilization) of the struggling animal, it may decrease the level of animal welfare instead of increasing it (Jordbruksverket, 2009). NSAIDs alone, even if administered 20 min prior the painful procedure, are not effective enough to reduce the acute pain associated with castration, and combinations of LA and NSAIDs are more effective than LA alone, especially for treating postoperative pain (Stafford and Mellor, 2005; Coetzee, 2011). Also, subcutaneous injections of LA or NSAIDs may be painful or irritating (Stafford and Mellor, 2005; EMEA, 2010; Viitasari et al., 2014).

Moreover, field anaesthesia of semi-domesticated reindeer is considered problematic: immobilized and anesthetized wildlife species frequently demonstrate serious side effects: hypothermia, hyperthermia, hypoxemia, and capture myopathy (Arnemo et al., 2014; Caulkett et al., 2015). Most drugs, including antagonists, used for sedating and anaesthetizing wild species are not approved for food-producing animals (Kreeger and Arnemo, 2012).

Our aim was firstly to investigate the effects of castration on reindeer behaviour, rectal temperature, and respiratory rate during the procedure, and secondly to explore how these factors are affected by handling, LA, and NSAIDs. Our hypothesis was that during clamp castration 1) reindeer show pain-related behaviours indicating that they feel pain, 2) local anaesthesia lessens the acute pain, 3) perioperatively administered NSAID doesn't affect the behaviour, and 4) prolonged handling adds to

stress shown as increased body temperature. To our knowledge, no previous research has been carried out concerning pain management in reindeer castration.

2. Materials and methods

Our present study is part of a larger project investigating pain alleviation and welfare in reindeer castration. Here we report the results from acute pain response. The study was approved by the Project Authorisation Board (ESAVI/36846/2019) and the Finnish Medicines Agency (Vetkl-nro 04/2019). The study was conducted in December 2019 at the Helsinki University Reindeer Research site in Savukoski, Lapland, Finland.

2.1. Animals and their management practices

A total of 55 clinically healthy, young, intact male reindeer, aged approximately 1.5 years (born May–June 2018) and weighing approximately 65–80 kg, were enrolled in the study. The reindeer were owned by the Kemin-Sompio reindeer herding cooperative and a single reindeer herder. Both owners signed an owner information consent form. After the rutting period ended and snow remained on the ground in November, we began capturing the previously chosen study animals, which had been marked during the round-ups held in November and December 2019. Our inclusion criteria were 1) a normal body condition and 2) no special remarks given during a clinical inspection (visual inspection, auscultation of the heart, lungs, and rumen) performed immediately after transferring the reindeer to our research site.

On the experimental site, the reindeer were released into a forest enclosure covering ca. 5 ha, with two smaller corrals for feeding and capturing. Animals were fed with commercial feed (Poro Balans 2, Kinnunen Mylly, Finland), dried hay, and lichen. The reindeer had soft, clean snow *ad libitum* to fulfil their water requirements (Soppela et al., 1988). The individuals adapted to enclosure conditions and feeding for a minimum of 10 days prior to the clinical trial.

2.2. Experimental design

The animals were divided into five treatment groups, $n = 11$ in each. The treatment groups were: 1) traditional castration (TRAD), 2) castration with meloxicam (NSAID), 3) castration with lidocaine (LA), 4) castration with meloxicam and lidocaine (LA+NSAID), and 5) non-castrated control (CONTROL). The target handling duration was one minute for groups 1 and 2 (short treatment) and six minutes for groups 3–5 (long treatment), recorded with a stopwatch. In the CONTROL group, the same animal served as a control for both the short and long treatments. A veterinarian administered the medications, and a professional reindeer herder performed the castrations, crushing the spermatocord with emasculator clamps from both sides for approximately 6–7 s per side.

On the trial day morning, animals were moved from a 5-hectare enclosure to a small wooden fenced corral. The weather condition was clear, followed by sleet later in the afternoon, with temperatures ranging between -1 and -4 °C. The application of the treatments was randomized (www.randomization.com) so that the treatments were spread evenly through the day in a random order. Reindeer herders, not aware of the following treatment, then caught the animals by hand in a random order and hence, the traditional *suopunki*(Fin)/*suohpan*(North Sami), a special rope, was not used for capture. The caught animals were then walked for 5 m to the treatment site. For the treatments, the animals were restrained in left lateral recumbency by two reindeer herders, as in a traditional routine castration procedure. The NSAID and LA+NSAID groups were administered a 2.5-ml subcutaneous injection of meloxicam (Metacam 20 mg/ml inject., Boehringer Ingelheim Vetmedica GmbH, Germany, with a target dose of 0.5–0.7 mg/kg) on their necks while still in a standing position, just before they were pushed over.

The TRAD and NSAID groups were castrated and released. The LA and LA+NSAID groups were treated with 10 ml lidocaine (Lidor vet. 20 mg/ml inject., Richter Pharma AG, Austria) injected and distributed to their spermatic cord and scrotal neck, castrated after 5 min of restraining, and released. The CONTROL group was held down in left lateral recumbency for 6 min and then released.

Rectal temperature (T) was measured (Beurer FT15/1 instant thermometer, Germany) from all animals immediately after pushing over (T0) and after castration/before releasing: TRAD and NSAID at the 1-minute point (T1); LA, LA+NSAID, and CONTROL at the 6-minute point (T6). In addition, rectal temperature was taken from LA, LA+NSAID, and CONTROL at the 1-minute point after LA was injected (T1) and in the middle of restraining (3-minute point (T3)). See Fig. 1 for the protocol.

After the trial, the animals were released back to the 5-hectare enclosure for the 28-day withdrawal period for meloxicam and lidocaine (cascade rule according to Directive 2004/28/EC) and then returned to their owners.

2.3. Behavioural assessment

Each reindeer's behaviour was filmed continuously during the castration procedure (Canon 5D MKIII, Canon Inc., Japan). Due to the short daylight time (polar night) at the end of December, we used three extra spotlights (Velvet Light 1, Velvet, Spain; Nordlight LED 2400, Blue Import AB, China; LED Energie Prof line 850, LED Energy Nordic AB, Sweden) in the trial enclosure.

As there are no previous studies documenting reindeer pain behaviours, we developed an ethogram as part of this study. The ethogram was first built by outlining the behaviours from ethograms created for other animal species (Molony et al., 1995; Dawkins, 2007). After this, two experienced ethologists, two veterinarians, and a veterinary student, all familiar with reindeer herding, defined the relevant pain-related behaviours and confirmed their expression and visibility by watching samples of the video recordings. Facial expressions, other than nostril movements, were excluded from the final ethogram, as video quality did not allow scoring such delicate movements. The following behaviours were included: head, neck, and leg movements, respiratory rate, panting, nostril closure, defecation, and abdominal muscle contraction. See definitions for behaviours in Table 1. The videos were then scored continuously with CowLog 3 (Pastell, 2016) in a randomized order by one blinded researcher, who was unaware of the treatments.

To examine the effects of the castration on behaviours, two observation periods were selected for each reindeer from the data for further analyses: prior to castration and castration. The prior to castration period was equal in length with the castration length of the same individual, but preceded castration. Castration was defined as the time from the beginning of castration (starting from the point when the performer's hands touch the testicles) and ending with the following rectal temperature measurement. Comparable observation periods were determined for the CONTROL group prior to T1 (short control) and T6 (long control), lasting the median duration of all castrations (24.6 s). In addition, the period of injecting local anaesthesia for the LA and

Table 1

Definitions for behaviours used for scoring clamp-castrated reindeer treated with a local anaesthesia and/or meloxicam, traditionally castrated reindeer, and the holding control.

Behaviour	Description
Defecating	Faeces coming out of rectum.
Single kick	Sudden angled, forward, or backward movement of a single leg flexing and straightening, max 1/s.
Several rapid kicks more than once/s, struggling	Movement of one or more legs in a struggling manner.
Leg flexion	Leg is bent close to the corpus, not kicking.
Leg straightening	Leg is straightened, over 45 degrees, not kicking.
Neck flexion	Neck is curved and muzzle is turned towards the sternum, not lifting head off the ground.
Neck straightening	Neck is straightened and muzzle is directed forward, not lifting head off the ground.
Head lifting (off the ground)	Upper part of neck, i.e., cheek and upper neck muscles, are lifted off the ground.
Other head movements	Minor changes in head position, head is not lifted off the ground
Head movement attempt	Restrained animal tries to move its head, which is visible as a contraction of the neck muscles and/or as movements by the holder.
Nostril closure	Nostrils are closed and form a line, lasting a minimum of 2 s
Panting	Breathing heavily with mouth open and rhythmical movements of head and/or chest.
Abdominal muscle contraction	Abdominal muscles retreat towards the spine while no respiration movements are visible.

LA+NSAID groups was defined as starting from the point when the performer's hand touches the testicles and ending with the following rectal temperature measurement. For all observation periods, the number of bouts was counted for single kicks, leg flexions and straightenings, defecating and single head movements. The number of bouts, total duration of bouts, and mean bout lengths were counted for the remaining behaviours. Respiration rate was also counted in 15-second sequences during the measurements of T0–T6, LA injection, and castration. Results are extrapolated to breaths/minute.

2.4. Statistical analysis

As most of the data did not follow normal distribution, nonparametric statistical procedures were applied. Before the analyses we calculated the differences between the temperature measurement points for each treatment separately. The differences between the treatments in behaviours, respiratory rates, and rectal temperatures were analysed with Kruskal-Wallis H tests with pairwise post-hoc comparisons. Friedman one-way repeated measure analysis of variance by rank with pairwise post-hoc tests and Wilcoxon pairwise tests were used to study the differences between sampling intervals on respiratory rates, temperatures, and behaviours (prior to and during castration) in the short and long treatments, respectively. We scored the number of reindeer that reached a rectal temperature of at least 39.5°C in T0–T6 and analysed the differences between treatments with chi squared analyses with Bonferroni correction for multiple tests to adjust significant values for all pairwise comparisons. Spearman's rank correlations were computed to

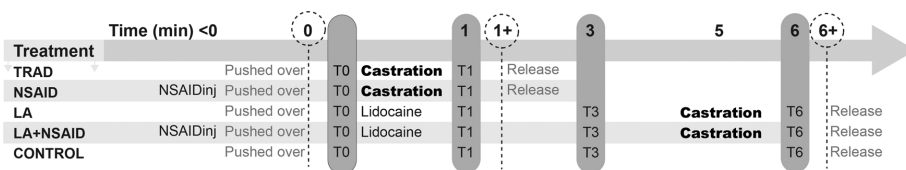


Fig. 1. Procedure protocol for treatments and control in a clamp castration trial for 55 reindeer divided in five groups: traditional castration (TRAD), castration with meloxicam (NSAID), castration with lidocaine local anaesthesia (LA), castration with lidocaine and meloxicam (LA+NSAID) and holding control (CONTROL). T0 to T6 = rectal temperature measurement points.

assess the relationship between T and respiratory rates.

Analyses were conducted using IBM SPSS Statistics (version 27.0, Armonk, NY, USA). P-values less than 0.05 were considered to indicate a significant difference and p-values below 0.1 a tendency for a difference.

3. Results

The actual median (interquartile range, IQR) treatment durations (from pushing over to release) for TRAD and NSAID groups were 70.00 (13.75) s and 74.00(19) s, respectively. Treatment durations varied between the longer treatments ($p < 0.05$): CONTROL differed statistically from LA and LA+NSAID; 391.00(6.00) s vs. 412.00(8.00) s and 408.00(14.25) s, respectively ($p < 0.001$ for both). The median (range) castration time was 24.6 (17.7–59.8) seconds and did not differ between the treatments.

3.1. Missing data

Due to technical problems, we excluded one TRAD reindeer from the rectal temperature analysis and one CONTROL reindeer from the behavioural and respiratory analysis. Due to poor visibility caused by sleet, we were unable to calculate the respiratory rates for 9/54 animals at T0 (3 CONTROL, 2 LA+NSAID, and 4 TRAD), 8/54 animals at T1 (2 from both CONTROL and NSAID, 1 LA, and 3 LA+NSAID), 3/23 animals at T3 (2 CONTROL and 1 LA+NSAID), and 4/23 animals at T6 (2 from both CONTROL and LA+NSAID).

3.2. Effects of castration, pain alleviation, and handling on rectal temperatures

A total of 25 animals reached a rectal temperature of 39.5°C. Of these, 2/10 were TRAD, 3/11 NSAID, 4/11 LA, 11/11 LA+NSAID, and 5/11 CONTROL. The number of LA+NSAID animals with $T \geq 39.5^\circ\text{C}$ tended to differ from LA at T1 (54.5% vs. 0%, $p = 0.05$) and differed from LA and CONTROL at T3 (100% vs. 18.2% and 27.3%, respectively, $p < 0.001$) and from LA at T6 (90.9% vs. 36.4%, $p < 0.02$). Five animals (all from LA+NSAID) reached a body temperature of at least 40.0°C at T6, the highest measured value being 40.5°C. One individual from the NSAID group reached 40°C at T0.

Rectal temperature values did not show statistically significant differences between the treatment groups at T0, T1, and T6. However, we found a difference between the treatments at T3 ($H(2) = 13.78$,

$p = 0.001$): rectal temperature was higher in the LA+NSAID group than in the LA or CONTROL groups ($p = 0.002$). Post-castration temperature was significantly different between long (T6) and short (T1) treatments ($H(3) = 11.30$, $p = 0.01$); LA+NSAID (= T6) was higher than TRAD and NSAID (= T1) ($p < 0.03$). No other differences between the groups were found (Fig. 2).

Overall, within each treatment rectal temperatures increased significantly from T0 onwards. There were significant changes between the first and last measures: In the short treatments the temperature increased significantly between T0 and T1 for both TRAD ($z = 2.13$, $p = 0.03$) and NSAID ($z = 2.53$, $p = 0.01$), and the increase between T0 and T6 was significant for all the long treatments: LA+NSAID, LA, and CONTROL ($\chi^2(3) = 20.04$, $\chi^2(3) = 14.58$, $\chi^2(3) = 17.14$, $p < 0.02$ for all), (Fig. 2).

3.3. Effects of castration, pain alleviation, and handling on behaviours and respiratory rates

3.3.1. Behaviours

Pain and defecation were seen occasionally throughout the treatments. We observed very few of the behaviours included in the ethogram prior to castration. Abdominal muscle contractions were not observed in any of the treatments prior to castration, and nostril closures were only occasional. We did not register differences prior to castration between short or long treatments, or when comparing treatments without CONTROL (Table 2).

However, during castration treatment differences were observed in the numbers, bout durations, and total durations of nostril closures in both the short ($H(2) = 20.27$, $H(2) = 17.87$, $H(2) = 17.87$, respectively, $p < 0.001$ for all) and long treatments ($H(2) = 11.45$, $p = 0.003$, $H(2) = 10.99$, $H(2) = 10.97$, respectively, $p = 0.004$ for all): the number and duration of nostril closures were smaller in CONTROL (short) than TRAD or NSAID, and in CONTROL (long) than LA and LA+NSAID ($p < 0.05$ for all). Similarly, there were treatment differences in the numbers, bout durations, and total durations of abdominal contractions, both in the short ($H(2) = 29.18$, $H(2) = 20.74$, and $H(2) = 20.74$, respectively, $p < 0.001$ for all) and long treatments ($H(2) = 21.09$, $H(2) = 19.47$, and $H(2) = 19.35$, $p < 0.001$ for all, respectively): the number and duration of abdominal contractions were greater in TRAD and NSAID than CONTROL (short), and in LA, and LA+NSAID compared to CONTROL (long)), respectively ($p < 0.05$ for all, Table 2).

During the local anaesthesia procedure (i.e., handling the testicles

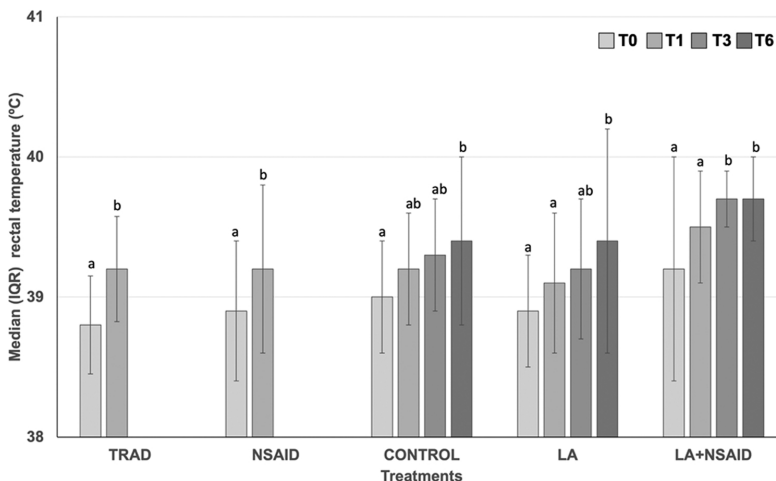


Fig. 2. Rectal temperatures measured two and four times (during short and long treatments, respectively) from 54 reindeer, aged approximately 1.5 years, during different treatments: traditional castration (TRAD), castration with meloxicam (NSAID), castration with lidocaine local anaesthesia (LA), castration with lidocaine and meloxicam (LA+NSAID), and holding control (CONTROL). Letters a and b mark statistically significant differences ($p < 0.05$) between the measurement points within each treatment.

Table 2
Observed median (interquartile range) of numbers and duration of behaviours prior to and during castration within five treatment groups: traditional castration (TRAD), castration with meloxicam (NSAID), castration with lidocaine local anaesthesia (LA), castration with lidocaine and meloxicam (LA+NSAID) and holding control (CONTROL). P-values < 0.05 are considered significant and marked with *, and p-values < 0.1 are considered with a tendency to be significant and marked with †.

Treatments	Parameters	Leg flexion		Leg straightening		Kicking more than once/sec		Head movements		Nostril closure		Abdominal contractions	
		Prior to castration	during castration	Prior to castration	during castration	Prior to castration	during castration	Prior to castration	during castration	Prior to castration	during castration	Prior to castration	during castration
CONTR short	No of bouts	0.00(0.25)	0.00(1.00)	0.00(0.25)	0.00(0.00)	0.00(0.00)	0.00(1.25) †	0.5(1.00)	2.00(1.5)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
	Bout duration					0.00(0.00)	0.00(1.74) †	0.96(2.4)	1.55(1.67)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
	Total duration					0.00(0.00)	0.00(2.38) †	0.96(2.66)	3.54(4.60)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
TRAD	No of bouts	0.00(1.00)	1.00(2.00)	0.00(0)	0.00(0.00)	0.00(0.00)	0.00(0.00)	1.00(2.00)	1.00(2.00)	0.00(0.00)	2.00(1.00)*	0.00(0.00)	2.00(0.00)*
	Bout duration					0.00(0.00)	0.00(0.00)	1.53(2.04)	1.44(2.05)	0.00(0.00)	4.16(1.64)*	0.00(0.00)	5.87(2.35)*
	Total duration					0.00(0.00)	0.00(0.00)	1.53(6.72)	1.44(3.26)	0.00(0.00)	7.57(9.07)*	0.00(0.00)	11.73(4.7)*
NSAID	No of bouts	0.00(0.00)	0.00(1.00)	0.00(0.00)	0.00(1.00)	0.00(0.00)	0.00(0.00)	0.00(5.00)	1.00(1.00)	0.00(0.00)	2.00(0.25)*	0.00(0.00)	2.00(0.00)*
	Bout duration					0.00(0.00)	0.00(0.00)	0.00(1.92)	1.82(1.00)	0.00(0.00)	4.82(3.28)*	0.00(0.00)	7.08(2.98)*
	Total duration					0.00(0.00)	0.00(0.00)	0.00(11.16)	1.96(2.91)	0.00(0.00)	9.63(7.53)*	0.00(0.00)	14.16(5.97)*
LA	No of bouts	0.00(0.00)	1.00(1.00)*	0.00(0.00)	1.00(1.00)*	0.00(0.00)	0.00(0.00)	0.00(0.00)	1.00(1.00)	0.00(0.00)	2.00(2.00)*	0.00(0.00)	2.00(1.00)*
	Bout duration					0.00(0.00)	0.00(0.00)	0.00(1.30)	0.00(1.96)	0.00(0.00)	4.96(6.24)*	0.00(0.00)	7.68(1.42)*
	Total duration					0.00(0.00)	0.00(0.00)	0.00(1.30)	0.00(1.96)	0.00(0.00)	6.56(12.48)*	0.00(0.00)	14.04(9.19)*
LA+NSAID	No of bouts	0.00(0.00)	1.00(1.00)†	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(0.00)	1.00(2.00)*	0.00(0.00)	2.00(1.00)*
	Bout duration					0.00(0.00)	0.00(2.76)	0.00(0.12)	0.00(1.05)	0.00(0.00)	6.12(7.15)*	0.00(0.00)	5.12(4.80)*
	Total duration					0.00(0.00)	0.00(2.76)	0.00(0.12)	0.00(3.44)	0.00(0.00)	6.16(1.4)*	0.00(0.00)	10.08(12.08)*
CONTR long	No of bouts	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.25)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
	Bout duration					0.00(0.00)	0.00(0.00)	0.00(0.52)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
	Total duration					0.00(0.00)	0.00(0.00)	0.00(1.05)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)

p < 0.05 = *, and p < 0.1 = †

and injecting lidocaine), neither abdominal muscle contractions nor nostril closures were observed. Some occasional head, neck, and leg movements or defecating were observed.

The number of leg flexions during castration differed between short treatments ($H(2) = 6.19$, $p = 0.045$): TRAD showed a higher number than NSAID ($p = 0.046$), while CONTROL did not differ from either. Also between the long treatments the number of leg flexion differed ($H(2) = 9.49$, $p = 0.01$): CONTROL showed fewer leg flexions than LA ($p < 0.05$) and tended to show fewer flexions than LA+NSAID ($p = 0.05$, Table 2).

When comparing intervals prior to and during castration within the long treatments the number of leg flexions increased in LA ($z = 2.46$, $p = 0.014$). In addition, during castration, the number of leg straightening also increased for LA compared with prior to castration ($z = 2.07$, $p < 0.05$). No differences were observed within short treatments.

3.3.2. Respiratory rates

The median respiratory rates decreased overall during the experiment within all treatments (Fig. 3). Within short treatments, the respiratory rates differed between T0 and T1 in NSAID ($z = -2.68$, $p = 0.007$) and tended to differ in TRAD ($z = -2.72$, $p < 0.07$). We also found differences within long treatments between measures T0–T6 in the CONTROL ($\chi^2(3) = 11.02$, $p = 0.012$) and LA ($\chi^2(3) = 21.02$, $p < 0.001$) groups, and a tendency in the LA+NSAID group ($\chi^2(3) = 7.56$, $p < 0.06$). The differences were between T0 and T3 in LA ($p < 0.001$), and between T0 and T6 in CONTROL ($p = 0.013$) and LA ($p = 0.002$).

Between treatments, the respiratory rates differed at T1 ($H(4) = 16.56$, $p = 0.002$): LA+NSAID being higher than NSAID ($p = 0.005$), TRAD ($p = 0.03$), and LA ($p = 0.02$). Respiratory rates also differed at T3 ($H(2) = 9.41$, $p = 0.009$): they were higher for LA+NSAID than LA ($p = 0.03$). No other differences were observed. The median (IQR) respiratory rates during injection of a local anaesthesia were 36(16) for LA ($n = 11$) and 44(26) for LA+NSAID ($n = 9$), not differing from T0 or T1.

Overall, when comparing the treatments without CONTROL during castration, the respiratory rates differed ($H(3) = 10.53$, $p = 0.015$): TRAD had the highest median respiratory rate differing from LA (38(40) vs. 32(20) breaths per minute, $n = 10$ and 11, respectively, $p = 0.024$). We also found differences when separately comparing the respiratory rates of the short and long treatments during castration ($H(2) = 9.89$, $p = 0.007$ and $H(2) = 6.07$, $p = 0.048$, respectively). Of the short treatments, NSAID had a higher respiratory rate during castration than CONTROL (40(12), $n = 11$, vs. 20(13) breaths per minute, $n = 10$, $p = 0.04$) and tended to have a higher rate than TRAD (34(40), $n = 10$, $p < 0.09$). Of the long treatments, LA tended to have a lower respiratory rate than CONTROL (20(12), $n = 11$ vs. 23(20) breaths per minute,

$n = 10$ respectively, $p < 0.06$), and LA+NSAID had 32(20) breaths per minute, not differing from either. We found positive correlations between T and respiratory rates at T1 and T3, $r_s(44) = 0.44$, $p = 0.004$ and $r_s(28) = 0.38$, $p = 0.04$, respectively.

4. Discussion

The present study suggests that reindeer express pain during clamp castration by closing their nostrils and contracting their abdominal muscles. There is a strong indication that a 5-minute effect time for local anaesthesia (lidocaine) failed to significantly reduce the pain-related behaviours. Moreover, a long handling time remarkably increases the rectal temperature of reindeer. As predicted, meloxicam administered subcutaneously perioperatively, immediately prior the procedure, did not reduce acute pain-related behaviours, but instead was associated to an increased body temperature possibly due to irritation in the injection site.

Nostril closures and abdominal muscle contractions were observed mainly twice per castrated individual: during castration, when the clamps were crushing the skin and spermatic cord both sides. This behaviour was not affected by local anaesthesia. Only occasional nostril closures were seen in three reindeer prior to castration. However, neither abdominal muscle contractions nor nostril closures were observed in non-castrated control animals or during either handling of the testicles or injecting lidocaine. Thus, these behaviours are likely to be interpreted as castration-related pain behaviours in reindeer.

The number of head and neck movements, kicking, panting, or defecating were not affected by castration or administered medication. Therefore, these behaviours can be interpreted as general struggling behaviours against manual immobilization, as described in Molony and Kent (1997). The role of leg flexions as a sign of pain remains partly unclear. An increase was observed in the number of leg flexions in local anaesthesia treated reindeer during castration versus CONTROL or prior to castration. However, this behaviour was also scored during the first minute in both traditionally castrated or with meloxicam treated and CONTROL animals, therefore, we interpret this relating to escape behaviours.

Our present study shows that the use of lidocaine local anaesthesia with a 5-minute waiting time is not a recommendable option to treat castration-related pain in reindeer round-ups for various reasons. The reindeer did not express pain behaviours at the time of lidocaine injection. However, even when an experienced veterinarian injected the LA, lidocaine did not reduce the number and duration of pain-related behavioural bouts observed in this study. This is in line with Mellema et al. (2007), who showed in lamb that local anaesthesia was not totally successful in reducing the immediate pain response. There are several

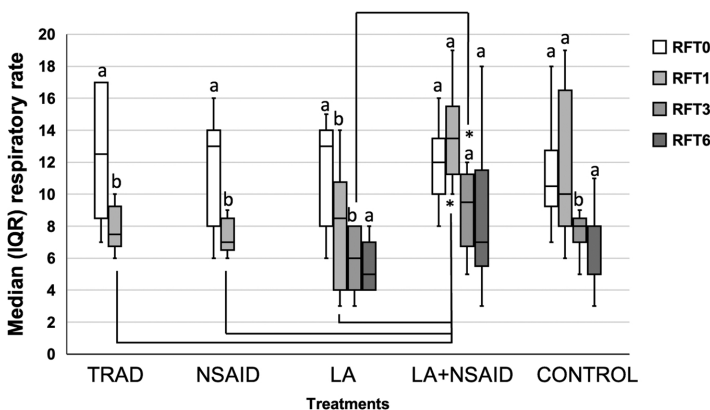


Fig. 3. Median (IQR) respiratory frequency (RF breaths/min) during T0 to T6 in clamp-castrated reindeer in five treatment groups: traditional castration (TRAD), castration with meloxicam (NSAID), castration with lidocaine local anaesthesia (LA), castration with lidocaine and meloxicam (LA+NSAID) and holding control (CONTROL). Differing letters above columns indicate significant differences ($p < 0.05$) between treatments at a specific measurement point, whereas asterisk (*) and black lines illustrate differences ($p < 0.05$) between the measurement points within treatments.

possible causes: first, our waiting time may not have been long enough. Coetzee (2011) indicated that the waiting time used in various calve castration trials was 5–20 min after local anaesthesia before castration with Burdizzo clamps was performed, even though the onset for lidocaine is quite rapid, 2–5 min (Coetzee, 2013). This is in line with Weinberg et al. (2015) who reviewed that the speed of onset of lignocaine is 5–15 min for peripheral nerve blockage and 1–5 min for local infiltration. Secondly, the area affected by injected lidocaine may have been too restricted. Noteworthy here is that this study was not even performed in the hectic field conditions of a reindeer round-up. Absorption of lignocaine (lidocaine) is dependent upon the total dose administered (Weinberg et al., 2015) and the dosage we used was calculated according to the safety level of the drug. Thirdly, it is also possible that the behaviours observed here, treated as binary variables, were not sensitive enough to measure how reindeer experience the strength of pain caused by castration clamps. Nevertheless, the increased handling duration was strongly associated with elevated rectal temperatures already with the 5-minute waiting time used in this study, also with the non-castrated control animals, indicating alarming capture and restraint stress. This does not rule out the presumable benefit of both lidocaine and meloxicam, if administered 20–30 min prior the procedure. Catching an animal twice is an additional major stress factor, but applicable especially with tamed tourist reindeer or reindeer otherwise more used to handling and human presence, in other than hectic great round-up situations.

However, when analysing the respiratory frequencies, we did observe some effects of local anaesthesia that may illustrate partial pain relief; during castration, TRAD had the highest respiratory rate differing from LA. All treatments affected the respiratory rate, they were higher than the reference values of 23–36 (lying vs. standing) breaths/min (White and Yousef, 1978), but mental and physical stress have also been shown to increase respiration frequency, even up to > 250 breaths/min under experimental conditions (Blix et al., 2011). It is noteworthy that respiration rate during castration is confounded by nostril closure and abdominal muscle contraction, which basically mean an individual holding its breath, and therefore these behaviours may affect the respiratory rate.

In this study, respiration rate also correlated with a rise in rectal temperature within the first 3 min, which may confirm previous results about respiratory rate being connected to not only mental and physical stress but also to hyperthermia (Lian et al., 2018). However, the association is not as straightforward, as rectal temperatures overall continued rising during the 6-minute observation time, while respiration rates decreased.

Our present study findings on increased rectal temperatures during handling indicate that the seasonal timing and treatment method of reindeer castration should be carefully considered, to avoid any extra stress. Reindeer have extremely thick and insulated winter hair, and they are therefore very susceptible to stress-induced body temperature increases (Lian et al., 2018), which was also indicative in this study. Heat stress in reindeer is highly related to capture method, with additive effects from physiological and mental stressors, such as fear, metabolic heat from muscular exertion, anaesthetic drugs, or high ambient temperature (Lian et al., 2018). The normal body temperature for resting reindeer is $38.6 \pm 0.2^\circ\text{C}$ (Blix et al., 2011), and rectal temperature may be even lower than the core body temperature (Ozeki et al., 2014). Signs of heat stress begin at a brain temperature of 39.0°C whereas the threshold for selective brain cooling during exercise is elevated to 39.5°C (Blix et al., 2011). Even though the clinical point for hyperthermia ($\geq 2^\circ\text{C}$ greater than normal) (Arnemo, 2014) is mainly higher than the rectal temperatures measured in our study, 11% of our study animals reached temperatures of at least 40.0°C , revealing a significant restraint induced stress.

The longer handling time due to 5-minute waiting in long treatment groups caused a significant rise in temperature, especially in reindeer that were administered both local anaesthesia and NSAID injections.

However, this same effect was not seen in reindeer administered only a local anaesthesia. The irritation caused by the subcutaneous meloxicam injection (EMA, 2010; Guo et al., 2021) may possibly have added to the stress caused by restriction. In the short treatment with NSAID, this irritating effect may have been shrouded by other simultaneous actions. Even though the initial rectal temperatures did not differ between treatments, LA+NSAID group had numerically slightly higher temperatures, therefore, we cannot rule out that it might have affected the results.

The longer-term benefits of lidocaine and NSAIDs are yet to be shown. Additional research is needed with more delicate measures and for various pain alleviation alternatives in reindeer. We only measured the signs of pain during castration itself. It is at least as important to define signs of post-procedure pain and find some proper means of alleviating it during the days following castration. We have recently shown (Nurmi et al., 2022) that oral meloxicam is a promising option when treating reindeer, hence, avoiding the assumed irritation caused by subcutaneous meloxicam injection is possible. The current Finnish Government proposal for Animal Welfare Legislation (VN/12277/2021) prohibits painful procedures without the use of analgesia. However, unless effective treatment can be initiated, in cases of acute distress, the immediate release of the reindeer should be targeted (Lian et al., 2018) until we have suitable means to properly alleviate acute pain. Sedation and anaesthesia have risks, especially in hectic reindeer round-up conditions during the cold winter period. These animals are mostly released back to the wilderness immediately after round-ups. It would be especially important to be able to antagonize anaesthetic drugs for faster recovery of respiratory depression and thermoregulation (Arnemo et al., 2014), and therefore the approval of antagonists for production animal use would be an animal welfare -friendly solution. Epidural anaesthesia is an interesting option but also problematic in winter if it decreases a reindeer's movement ability for too long a time. When possible, castrating these animals in a standing position could lessen the stress caused by restraining. Avoiding castration is not always possible, however, as slaughtering males intact before or after the rut and rehabilitation is an option in certain circumstances and areas. Immunocastration may provide some possibilities in the future, but the current protocol (Zamaratskaia et al., 2008; EMA, 2009) requires repeated injections five months apart, which is unsuitable in most reindeer herding areas.

5. Conclusions

Reindeer as prey animals show modest castration pain, in this study mainly by contracting their abdominal muscles and closing their nostrils. Local lidocaine anaesthesia failed to significantly reduce pain-related behaviours, and longer handling times meanwhile caused a significant rise in rectal temperature, especially if combined with other stressors such as NSAID injection. Further research is needed with more delicate measures of pain related to castration and the proper means of pain alleviation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The effect of a non-steroidal anti-inflammatory drug on the distance travelled by reindeer (*Rangifer tarandus tarandus*) in the wilderness after clamp castration - a pilot study

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Abstract

Background: During seasonal round-ups, free-grazing reindeer are gathered from natural pastures. Reindeer bulls removed from breeding are clamp castrated, traditionally without analgesia, and then released back into the wild. The new Finnish Animal Welfare Act requires the use of analgesia in painful procedures. A single dose of the non-steroidal anti-inflammatory drug meloxicam may maintain therapeutic plasma concentrations for 2–3 days in reindeer. No studies have been conducted on the effect of meloxicam on the behaviour of castrated reindeer in the wild after the procedure. We installed GPS collars on 16 male reindeer (5y, 130–160 kg), chosen to be castrated as a standard procedure during the round-up. Of these, eight were randomly selected to receive approximately 0.5 mg/kg of meloxicam subcutaneously (NSAID) and eight received no analgesia (TRAD). From the GPS data, we calculated the daily distances travelled by the reindeer during the three days after castration and analysed the differences between the treatments using a GEE model. Fixed factors were treatment (NSAID or TRAD), days (1–3) and hours, and the interactions between these variables.

Results: Overall, the mean $\pm$ sem daily distances travelled by NSAID and TRAD reindeer did not differ (6.60 ± 0.67 km vs. 8.60 ± 1.54 km) but the days differed: reindeer travelled longer daily and hourly distances on the 1st than the 3rd day, and TRADs travelled longer distances than NSAIDs during the first day after castration (11.67 ± 2.25 km vs. 7.08 ± 0.61 km), with no difference observed on the 2nd or 3rd day (10.19 ± 3.87 km vs. 6.59 ± 0.85 km and 5.35 ± 0.39 km vs. 6.17 ± 0.70 m, respectively). Day- and night-time distances did not differ on the 1st day, but the daytime distances travelled on the 2nd and 3rd days were longer than during respective nights. The travelled daytime distances remained steady while night-time distances decreased. The daily rhythm was particularly broken with the more restless TRAD group.

Conclusions: Meloxicam may reduce restlessness in castrated reindeer in the wild for two days after castration. Further studies are needed on the use of analgesia and GPS collars for pain monitoring in freely grazing reindeer.

Background

An estimated 2500–4000 reindeer are clamp castrated annually in the reindeer herding area in Northern Finland. Traditionally, the operation has mostly been performed without pain alleviation measures. Free-grazing reindeer herds are gathered from the wilderness into seasonal round-up enclosures. Old males removed from breeding are clamp castrated and released back to their natural pastures in the wild. Now, the Animal Welfare Legislation in Finland has been reformed; pain alleviation is mandatory in painful procedures from 2024 onward [1].

Research on other ruminants indisputably demonstrates that clamp castration is painful [2,5]. Animals move less or stand more after castration and show characteristic pain-related stiff movement or standing postures [6,9]. Moreover, untreated pain manifests as restlessness and increases in locomotor activity [6] and as alterations in resting and sleeping behaviours [10,12]. However, compared to various castration methods, successful clamp castration seems to cause the shortest afterpain period to young bovine calves [13]. The first few hours are the worst [14, 2], and scrotal swelling increases for approximately two days, after which symptoms of pain and swelling usually disappear within 15 days [13, 15]. Unlike other ruminants, reindeer are castrated as adults.

Prey animals are generally very stoic to pain, as expressing pain does not give them a biological advantage [8]. In line with this, we have previously reported that reindeer show subtle pain behaviours at the moment of acute pain caused by clamps crushing the tissue [16]. Postoperative castration pain can be treated with nonsteroidal anti-inflammatories (NSAIDs) [17], such as meloxicam; a PGE2 synthesis inhibitor with anti-inflammatory, anti-pyretic, and analgetic properties [18]. We have also recently shown that a single dose of meloxicam (0.5 mg/kg intravenously or orally) has the potential to maintain the therapeutic concentration in reindeer plasma determined in other species for up to three days [19]. However, no studies exist on how NSAIDs alter reindeer behaviour, such as activity in the wild in the first days after clamp castration.

Reindeer maintain a circadian rhythm throughout the year, with periods of ultradian activity and inactivity alternating over a 24-hour period [20,–21]. Dawn and dusk divide reindeer activity into day and night, with daytime activity predominating and activity peaks ranging from 4–6 in winter to 6–9 in summer [22]. Untreated pain can alter the activity patterns of various animal species [12], but to date no studies have been conducted on reindeer.

In recent years, advanced tracking techniques, such as Global Positioning System (GPS), have proven useful in livestock management and in improving animal welfare [23], and it is currently widely used in reindeer husbandry. Human influence on the research results of behavioural studies can also be minimized by utilizing GPS [24,–25]. GPS collars can be used to track animal movements over

large areas [23], and animal speed has been shown to be a valid measure of activity [26]. GPS data can also be used to determine circadian rhythms and changes in activity under various conditions [23].

Our aim was to study the effect of a NSAID (meloxicam) on the activity of newly castrated reindeer for three days in the wilderness. Our hypothesis was that pain affects the activity habits of reindeer.

Methods

The study was approved by the University of Helsinki Viikki Campus Research Ethics Committee (Statement 16/2020) and the Finnish Medicines Agency (Vetkl-nro 04/2019) and was performed in the Naltio round-up enclosure (67°N 29°E) in Savukoski, Finland on October 5th, 2020.

Animals and management

We enrolled 16 healthy reindeer that the reindeer owners chose to be castrated as a part of routine reindeer herding at the Kemin-Sompio reindeer co-operative round-up. The randomly chosen reindeer were at least 5 years of age, and their estimated weights ranged between 130 and 160 kgs. All owners signed an informed consent.

As a standard herding procedure, approximately 1300 reindeer were gathered with helicopter and all-terrain vehicle assistance from the wilderness to Naltio round-up enclosure for five days prior to round-up. The round-up area has a larger forest enclosure of about 960 hectares, with sufficient space and natural pastures to feed 5000 reindeer and brooks and springs for water supply.

During round-up, the reindeer were moved in approximately 100-animal batches to a circular round-up corral in which the animals were identified and counted, their health statuses were checked, the animals for slaughter were separated from the others, unmarked calves were ear-marked, males removed from breeding were castrated, and the breeding reindeer were treated with the anthelmintic drug ivermectin 0.2 mg/kg liveweight subcutaneously (Virbamec 10mg/mL, Virbac, France). After these measures, the reindeer were released into a new, spacious enclosure to await release into the wild after separation. The round-up lasted approximately from 11–17, after which the animals were released from the enclosures into the wild. The weather was clear, + 5 °C during the treatment day and remained stable during the following three days, fluctuating mildly from slightly above 0 °C to a maximum of + 10 °C (measured from three weather observation stations located on the corners of the wilderness area).

Study design

The 16 study animals were randomly allocated into two eight-animal groups: the NSAID (castrated and treated with a non-steroidal anti-inflammatory drug) group was treated with a subcutaneous injection of 4 mL meloxicam (Metacam 20 mg/mL

inject., Boehringer Ingelheim Vetmedica GmbH, Germany) and the TRAD (traditional non-medicated castration) group did not receive analgesia. Animals were caught by hand by 2–3 reindeer herders and immobilized for castration by bringing them down to the ground.

Castration was performed with emasculator clamps, crushing the seminal cord for 6–7 seconds per side. All animals were given an ivermectin injection and NSAID-treated animals were injected with meloxicam; all injections were administered subcutaneously on the neck before the animals were allowed to stand up.

Registering and data handling

Each study animal was equipped with an Ultracom GPS tracker (Tracker Ltd, Oulu, Finland) when brought down for castration. The trackers were pre-set to provide locations twice an hour. GPS tracker data were transferred into geographical map points. The distances between two sequential measurement points were analysed using the following formula:

$$ACOS(COS(RADIANS(90 - L1)) * COS(RADIANS(90 - L2)) + SIN(RADIANS(90 - L1)) * SIN(RADIANS(90 - L2)) * COS(RADIANS(D1 - D2))) * 6371$$

in which L1 = latitude 1, L2 = latitude 2, D1 = longitude 1, and D2 = longitude 2.

Three of the GPSs were out of satellite reach for 1–2 hours during the first day and for two hours on days 2 and 3. We used a Bayesian statistical method for imputing the missing data [27].

Statistical analysis

We calculated the average daily and hourly distances travelled by reindeer after castration and the mean hourly distances travelled during daytime (7–17) and night-time (17–7) hours. As the data did not follow a normal distribution, we analysed the differences between the treatments (NSAID and TRAD) using the Generalized Estimating Equations Model (GEE), which is suitable for processing repeated measures data when the data distribution is non-normal [28]. In the model, the factors used in analysing average mean daily distances travelled were the days after castration (1–3), the treatments (TRAD and NSAID), and their interactions. Accordingly, the factors used in the model analysing differences in hourly travelled distances over three days were treatments, days, daily hours, and their interactions. The model that analysed the differences in day-night activity had treatments, days, and day–night times, along with their interactions, as factors. In all models, we employed a log-link function, utilized unstructured correlation structures, employed robust estimators for the covariance matrix, and applied Fisher parameter estimation for maximum likelihood estimates. Given the nature of GEE, explicit degrees of freedom for the model cannot be provided.

The analyses were performed utilizing IBM SPSS Statistics (version 29.0, Armonk, NY, USA), and significance was determined at a threshold of $P < 0.05$.

Results

Overall, the reindeer travelled 7.53 ± 0.75 kilometres per day during the three days. The variation was high, and no significant differences ($P > 0.1$) were observed between the NSAID and TRAD reindeer in the mean $\pm$ sem overall distances travelled daily during the three days (6.60 ± 0.57 km vs. 8.60 ± 1.54 km, respectively). The reindeer moved more or less singly but within the same geographical area over the three measurement days (Fig. 1).

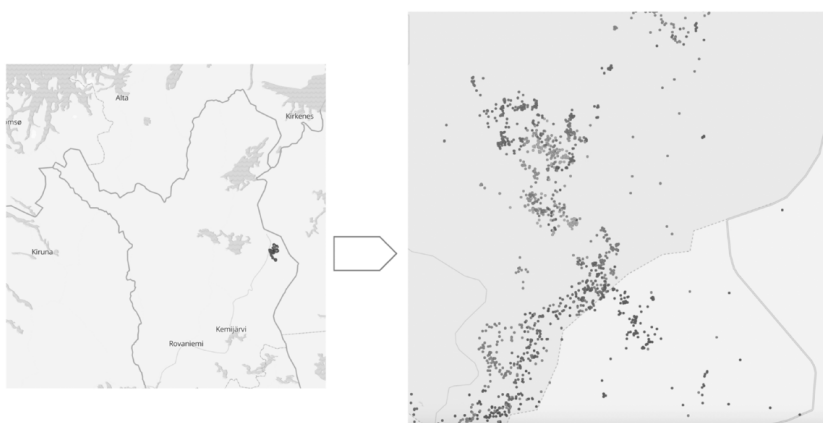


Figure 1 Distances travelled by 16 study reindeer on a map during three days after castration. Each point corresponds to the GPS position of one reindeer measured every half an hour. Individual reindeer are marked in different colours: Red colours belong to TRAD and green/blue to NSAID treatments. ©Flourish, ©OpenMapTiles ©OpenStreetMap contributors. Figure A shows an overview of the area where the reindeer are travelling and Figure B shows a close-up of the reindeer movements.

However, the days differed from each other ($P < 0.001$); reindeer travelled longer distances on the 1st than on the 3rd day after castration (9.09 ± 0.96 km vs. 5.75 ± 0.39 km, $P < 0.001$), while distances travelled on the 2nd day (8.20 ± 1.64 km) did not differ from either the 1st or the 3rd day. Moreover, we found a significant interaction between treatments and days ($P = 0.01$): TRAD reindeer travelled longer distances than NSAID reindeer during the first day after castration (11.67 ± 2.25 km vs. 7.08 ± 0.61 km, $P < 0.05$), with no differences observed on the 2nd and 3rd day (10.19 ± 3.87 km vs. 6.59 ± 0.85 km and 5.35 ± 0.39 km vs. 6.17 ± 0.70 m, respectively). And further, distances travelled by NSAID reindeer did not differ between the days, but the activity of TRAD reindeer decreased over three days (Fig. 2).

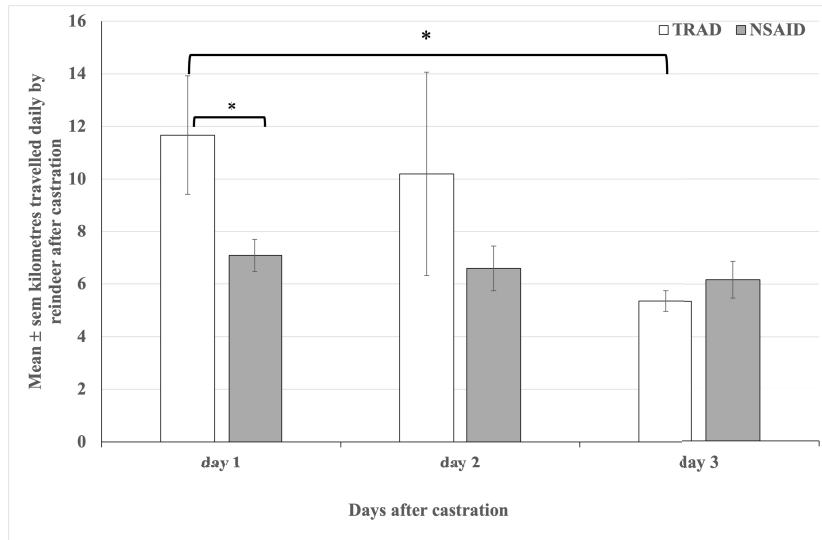


Figure 2 The overall mean ± sem kilometres travelled by the newly clamp-castrated reindeer in the wilderness. NSAID reindeer (n=8) were injected with 0.5 mg/kg meloxicam subcutaneously and TRAD reindeer (n=8) were castrated without analgesia. The whiskers and asterisks mark statistically significant differences.

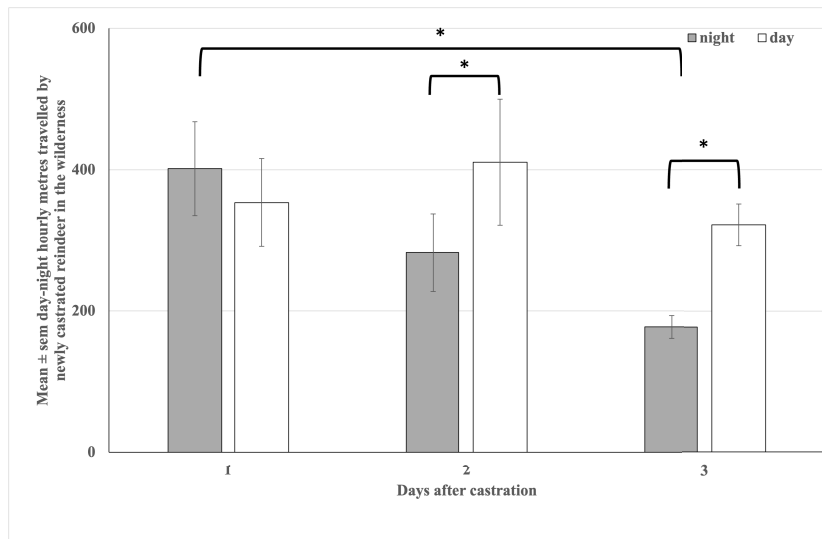


Figure 3 Mean ± sem day-night hourly metres travelled by the newly clamp-castrated reindeer in the wilderness during three days. NSAID (n=8) was injected with 0.5 mg/kg meloxicam subcutaneously and TRAD (n=8) was castrated without analgesia. Statistically significant differences are marked with whiskers and asterisks.

The overall hourly distance travelled by the reindeer was 272.12 ± 20.72 metres during the 3 days, which did not differ statistically between TRAD and NSAID

reindeer (302.31 ± 39.91 km vs. 244.94 ± 18.61 m, respectively). However, we found differences between the days and hours ($P < 0.001$); overall, reindeer tended to travel longer hourly distances during the 1st rather than the 2nd day (345.78 ± 31.38 m vs. 276.39 ± 39.44 , $P = 0.05$) and travelled shorter distances on the 3rd (210.85 ± 13.51 m) than on the 1st day ($P < 0.001$).

The total hourly daytime and night-time distances travelled by reindeer differed ($P < 0.005$); the reindeer travelled shorter distances during night-time than during daytime (271.89 ± 24.88 m vs. 360.26 ± 46.06 m, $P = 0.02$). We observed no overall differences between the treatments, but there was an interaction between the days and the day-night phases: the total distances travelled by day and by night did not differ on the 1st day, but longer daytime distances were travelled on days 2 and 3 than during respective nights. The travelled daytime distances remained steady, but the night-time distances decreased (Fig. 3). The difference between days and hours was respective to Fig. 2 (data not shown).

Overall, the reindeer travelled the longest hourly distances during daytime hours (06–15). Moreover, significant interactions were observed between treatments, days, and hours ($P < 0.001$), showing that the daily rhythm was broken by the more restless TRAD group (Fig. 4). During the start of the first day after the restless first night, the NSAID group had a higher activity peak than the TRAD group between 8–11, otherwise TRAD reindeer travelled longer hourly distances, especially between 15–16. However, the variation was high in TRAD.

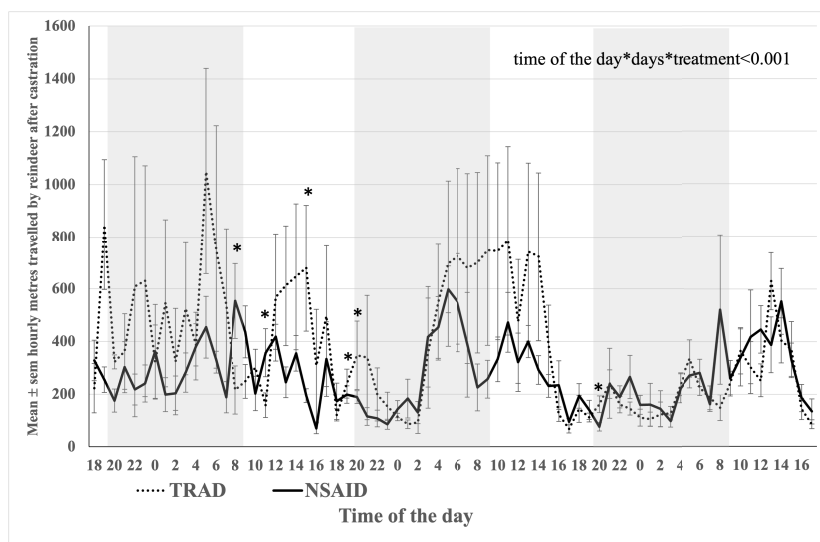


Figure 4 The overall mean $\pm$ sem hourly kilometres travelled by the newly clamp castrated reindeer in the wilderness for three days. The NSAID group ($n=8$) was injected with 0.5 mg/kg meloxicam subcutaneously and the TRAD group ($n=8$) was castrated without analgesia. The blue shading indicates night-time hours. Statistically significant differences are marked with asterisks.

Discussion

Here, we show, for the first time, that untreated pain increases the movement of castrated reindeer in the wild. The subcutaneously administered NSAID meloxicam reduces the activity of castrated reindeer in the first two days after castration compared to reindeer castrated traditionally without NSAIDs. The overall day-night activity difference disappeared during the 1st day after round-up and clamp castration but returned from the 2nd day onwards. GPS collars are promising devices for monitoring pain behaviour in free-ranging reindeer.

Immediately after the round-up and castration, reindeer from both treatments exhibited activity during the 1st night and lost their normal diurnal activity rhythm. The loss of diurnal rhythm may be attributed to several factors, including the stress of gathering and round-up, short-term irritation caused by the ivermectin injection, as reported by the manufacturer [29], and/or the potential tissue irritation caused by subcutaneous meloxicam, as suspected in our previous study [16]. Following the 1st night, the reindeer in both groups resumed a clear diurnal activity pattern, consistent with previous observations [22].

We found that clamp-castrated TRAD reindeer were particularly active during two days after castration, and their activity decreased to the same level as NSAID reindeer on the third day after castration. After the first restless night, the TRAD group moved less than the NSAID group the following morning. Overall, the NSAID group maintained a more consistent level of movement over the first three days. Our results are in line with previous findings showing that post-castration pain is the worst during the first two days after the procedure [14, 2, 13]. The subcutaneously administered meloxicam appeared to decrease reindeer activity compared to untreated reindeer, especially during the first two days. Although only the intravenous and oral routes of administration have been studied in reindeer thus far, serum concentrations in reindeer remained at the presumed therapeutic level for 36–72 h regardless of the route of administration [19]. Also, in goats and sheep, both subcutaneous and intramuscular injections of meloxicam achieved rather rapid high plasma concentrations at therapeutic levels for up to 48 h [30,31].

Notably, due to our study being a pilot experiment, we did not have a non-castrated control group. Moreover, the rut was still on, and fitting GPS collars on fighting bulls would have created an animal welfare concern. Thus, our results should be interpreted with caution, as increased activity after a painful procedure could either be a positive or a negative sign [32]. However, reindeer in general are a rather vigilant and flight-reactive species [33–35], and thus increased activity in the wilderness because of pain is not unexpected but merely logical and in line with other studies on pain and activity [36]. Moreover, comparisons with GPS data from the Natural Resources Institute Finland (LUKE) reindeer, collected in the same area and season, validate our NSAID group findings, although for female reindeer, the 4-km distances travelled per day by the LUKE reindeer [37] were more in-line

with our NSAID reindeer. Our study reindeer mainly moved separately. Social behaviours may play less of a role during the autumn rut with males, even though they tend to form separate groups during summer [38].

We faced some challenges in our data collection, dealing with a semi-domesticated species whose individuals are prone to ridding themselves of any foreign objects attached to them, and finally, with animals grazing in the great wilderness close to the border of Russia. Several blind spots were observed when collecting GPS signals and reindeer locations, and these blind spots increased the further our study animals grazed from the round-up enclosure. Despite the prominent variation and missing data, the current analysis was sensitive and sufficient for answering our research question, showing that reindeer appear to benefit from NSAIDs administered at the time of clamp castration.

Conclusions

NSAIDs contribute to the welfare of castrated reindeer, as a single dose of meloxicam reduces their activity, potentially a sign of reduced agitation in the wild. Further studies are needed on the efficacy, toxicity, therapeutic levels, and optimal routes of administration of various NSAIDs to reindeer, to establish the best practice in using analgesia in reindeer castration.

Declarations

Authors' contributions

HN: the conception and design of the study, data acquisition, analysis and interpretation of data, manuscript preparation, funding acquisition; **LH:** the conception and design of the study, data acquisition, analysis and interpretation of data, manuscript preparation, funding acquisition. **SL:** study design, data acquisition and interpretation, manuscript preparation, **AV:** study design, data acquisition and interpretation, manuscript preparation; All authors have read and approved the final version of the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The studies were approved by the University of Helsinki Viikki Campus Research Ethics Committee (Statement 16/2020) and the Finnish Medicines Agency (Vetkl-nro 04/2019).

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