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Role of porcine models in translational pain research – a systematic review

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Abstract

Translating basic pain research from rodents to humans has proven to be a challenging task. Efforts have been made to develop preclinical large animal models of pain, such as the pig. However, no consistent overview and comparison of pig models of pain are currently available. Therefore, in this review, our primary aim was to identify the available pig models in pain research and compare these models in terms of intensity and duration. Firstly, we systematically searched Proquest, Scopus and Web of Science and compared the duration for which the pigs were significantly sensitized as well as the intensity of mechanical sensitization. We searched models within the specific field of pain and adjacent fields in which pain induction or assessment is relevant, such as pig production. Secondly, we compared assessment methodologies in surrogate pain models in humans and pigs to identify areas of overlap and possible improvement. Based on the literature search, 23 types of porcine pain models were identified; 13 of which could be compared quantitatively. The induced sensitization lasted from hours to months and intensities ranged from insignificant to the maximum attainable. We also found a near to complete overlap of assessment methodologies between human and pig models within the area of peripheral neurophysiology, which allows for direct comparison of results obtained in the two species. In spite of this overlap, further development of pain assessment methodologies is still needed. We suggest that central nervous system electrophysiology, such as electroencephalography, electrocorticography or intracortical recordings, may pave the way for future objective pain assessment.

Introduction

Chronic pain affects the lives of approximately 20% of the population, their family members and society[1–3]. Yet no adequate treatment is available and it is common practice to relieve pain symptoms with pharmacological agents[1]. Developing an effective and valid translational animal model of pain is a challenging task, and pharmaceutical trials often fail translating promising results from rodents to humans[4–7]. Differences in the pain system of rodents and humans are believed to contribute to these failures[4,5]. Based on the subjective nature of pain[8], higher order cortical processing is required for pain perception**,** and individual context and social interaction are critically modulating the subjective pain experience. Therefore, the assessment of pain experience in humans is often based on patient-reported outcomes. The experienced pain intensity is typically tested using a numerical or visual analog scale, while the intensity, location and the quality of the pain sensation can be assessed with the McGill pain questionnaire[9]. Pain assessment in animals predominantly consists of testing stimulus-evoked nociception or nocifensive behavior rather than spontaneous pain-related behavior[5,7,10].

For a translational pain model to be valid, it must to some extent replicate the pathophysiological and psychological characteristics of the human disease and it should be able to predict the effectivity of potential treatments[10]. These aims are challenging as psychological characteristics are hard to translate and the pathophysiology of human chronic pain conditions is unknown, with no consensus on where in the neuraxis the critical changes occur[11]. However, animal models ─ rodent models in particular ─ have successfully helped to unravel the molecular mechanisms and circuits of pain processing[12–17]. Therefore, improving translation from animal models to humans by selecting suitable approaches for each individual question, while taking into account the limitations of each model, seems to be the way forward.

In rodents, a variety of pain models are well established and characterized[5,14–20]. Moreover, genetic modification allows for unique mechanistic studies[21] and new test paradigms such as place preference tests, pain-depressed operant behavior or facial expression[22] are constantly developed to improve

translatability. However, there are limitations related to species differences particularly in the central nervous system[23], the skin structure[24], the lack of naturally occurring chronic pain conditions and the limited lifespan, as well as practical problems such as interspecies scaling[25,26]. On the other hand, pigs show greater similarity to humans in terms of sequency homology, metabolism, digestive system[27], central nervous system[28–30], peripheral nociceptive and non-nociceptive fiber classes and axonal excitability[27,31–33], but also similarity in body size[27]. Moreover, pigs respond similarly to humans to different pharmaceuticals, which could be expected given their great genetic sequence homology with humans[5]. For example, aprepitant ─ a drug that showed promising results in rodents ─ later failed to translate to humans in clinical trials[4,34], whereas HTX-011 ─ a dual-acting local anesthetic that contains two active ingredients ─ showed a synergistic effect in pigs, which was replicated in human clinical trials[35,36]. Despite these encouraging results and the similarities shared with humans, the pig is not commonly used in pain research.

Our primary aim was to identify the different available porcine models that could be used as pain models. Therefore, we focused not only on models that are already available within pain research but also in other disciplines such as pig production and research in other diseases. The identified porcine models were compared qualitatively and quantitatively in terms of intensity and duration. We defined 'intensity' based on the outcome measure most comparable between studies, i.e. mechanical sensitization, and we defined 'duration' as the period in which the pigs were mechanically sensitized. Given that assessment is a key element in evaluation and interpretation of the pain in translational pain research, we also extracted information on pain assessment methods used in the porcine models and compared these with assessment methods used in humans.

Recent reviews on pain in pigs mainly focused on the welfare implications of management procedures in pig production[37–40] or discussed pain assessment methodologies and practices in large animals[27,41,42]. However, no consistent overview and comparison of available pig models of pain is currently available. We believe that this knowledge will assist in improving the translatability of animal pain research to human applications.

Methods

Literature search for porcine pain models. We performed a systematic search in Proquest, Scopus and Web of Science to identify all relevant literature concerning pain in porcine models. The protocol was not registered. It was anticipated that the number of pain studies and models in pigs would be limited. Keywords and criteria used for the search are listed in box. 1.

Qualitative and quantitiative comparison of the models. Given that our primary aim was to identify the currently available pain models in pigs, we retrieved all information from the included literature and first performed a qualitative comparison between models. However, to compare the duration and intensity of the different pain models, a common outcome measure was required. Although 31 studies assessed a behavioral response, no standardized assessment was used. Therefore, these outcome measures were not suitable for our quantitative comparison (Dzikamunhenga et al.[38] reported the same problem). Instead, we selected mechanical sensitization for this purpose since this methodology was used in 18 studies. The intensity was calculated as the decrease in the withdrawal threshold from baseline. This resulted in a scale ranging from 0 to 1; 0 reflecting baseline and 1 reflecting a 100% decrease in withdrawal threshold. When more than one study described the same model, the intensity was a weighted combination of the results from the different studies.

Bias assessement. To evaluate the risk of bias, we assessed all articles in terms of blinding, use of a control group, randomization and numbers of animals used (Supplementary Table 1). However, none of the studies were excluded due to risk of bias because the purpose of this review was to identify the pain models available. We considered the effect of bias on the cumulative evidence to be minor given that only a few studies are currently available for each model.

Literature search for human pain models. The most recent reviews on pain assessment all conclude that the pain assessment methods currently used in pigs are insufficient[27,30,42,43]. To suggest relevant areas for improvement of pain assessment in pigs, we qualitatively compared pain assessment methods in pigs to those used in humans. A systematic search for reversible surrogate pain models (including nerve growth factor (NGF) injection, carrageenan injection, UV-B irradiation and capsaicin patch) in humans was carried out in Scopus. Titles were scanned to select relevant articles. This search was secondary and aimed at a qualitative comparison with porcine models. This search was not conducted as thoroughly as the search for porcine pain models and did not follow the Prisma guidelines.

Results overview

Using the defined search strategy, we identified 239 documents; 103 in Scopus, 56 in ProQuest, 23 in Web of Science and 57 from reference lists. A total of 147 full-text documents were retrieved and a total of 73 articles were included in the review (see Fig. 1). Reasons for exclusion were: study out of scope, methodological issue (i.e. pain not being an outcome measure), type of study (i.e. not peer-reviewed), duplicate or if the full-text was not available. The included research literature was grouped in four categories (Table 1): surrogate pain models, disease models, naturally occurring pain and pig production procedures, according to the reported purpose of the model. In the next section, we first present a qualitative overview of the different pain models grouped by category. We then compare the intensity and duration of the different porcine models and lastly compare the pain assessment methods between pig and human research.

Porcine pain models

Pain research in pigs started for the sake of relieving pain due to production procedures[44]. The pig was then used as a translational disease model[45] and has only recently gained interest as a translational model in pain (Fig. 2). The earliest studies in which pain was evoked were aimed at providing proper analgesia for pigs included in research[46–48]. In this section, we present an overview of the available pig models grouped into four categories (surrogate pain, pig production, natural pain and disease models). A summary of the different models is provided in Table 2.

Surrogate pain models. Surrogate pain models are models developed solely for the sake of studying pain, including pain mechanisms and treatments. A total of 20 research papers on surrogate pain models were identified and subdivided into surgical, inflammatory and irreversible pain models.

Surgical models

All the eight surgical pain studies identified had the purpose of testing pharmaceuticals for use in research pigs[46–50] or intended for human use[51–53]. As the focus of this review was on pain models and not on the effectiveness of pharmaceuticals to relieve the induced pain, we considered pain suppression by analgesics as an indication of the validity of the pain model.

Analgesic treatments were tested in pigs using implantation of an arterial catheter[47], laparotomy[50] or abdominal surgery[48] as surgical models. These studies showed increased levels of serum cortisol[48,50] and ACTH[50] in pigs during and immediately after surgery compared with baseline, and increased Fos-like-immunoreactive (Fos-LI) neurones in the dorsal horn in pigs undergoing surgery compared with sham pigs[50]. These levels could be reduced by administration of analgesics[50] to levels that were found at baseline or in the sham group. Malavasi et al. showed that active behavior was decreased at least two days after abdominal surgery but could be increased to the presurgical levels with morphine and fentanyl[48]. Meloxicam and paracetamol also improved behavioral scores compared to an untreated control group[47] The laparotomy model[50] was also used as a translational model to study the effect of morphine during surgery[49].

A 2014 study introduced a pig model of incisional pain as a post-operative pain model for efficacy testing of pharmaceutical compounds[51]. Two different incision models were tested: skin incision alone (SI) and combined skin and muscle incision and retraction (SMIR). Both models resulted in a large decrease in the withdrawal threshold (approximately 95%) compared to intact skin) up to one week after surgery[51–53] and withdrawal thresholds were slightly lower for SMIR than for SI[51]. Wilsey and Block found an increasing trend in the withdrawal thresholds from five days after SMIR[53] as opposed to Castel et al. who showed that at 7 days post-surgery, the withdrawal threshold of the incised skin was still lower compared with the intact skin of the same pig[51]. Morphine normalized the withdrawal thresholds completely for the SI, but not the SMIR model, while other local treatments resulted in an incomplete normalization[51,52]. SMIR resulted in higher spontaneous pain-related behavior than SI; morphine improved spontaneous behavior scores in both SI and SMIR pigs[51]. Spontaneous locomotor activity, which was measured in an open-field setup, was not affected in the SMIR model[53].

Inflammatory models

Four types of inflammatory pain models were identified in the literature search: NGF injection[54–57], UV-B irradiation[58,59], injection of carrageenan[60,61] and application of a capsaicin patch[62]. The NGF model has been used most frequently, mainly with the purpose of understanding the neurophysiological processes underlying hyperalgesia at the peripheral level and studying how the pig can serve as a translational model in that regard. Although the majority of the studies only looked at nociceptor and peripheral nerve activation, some studies were also coupled with human experiments[55,56], making it possible to link nociceptor and peripheral nerve fiber activation to the subjective sensation of pain. Sensitization was detected in humans by increased pain ratings following electrical stimulation, which reached a maximum three weeks after NGF injection[55]. Similar results were obtained in pigs, which showed a decrease in mechanical activation thresholds three weeks after injection of NGF[56]. Mechanical sensitization might also be explained by a decrease in activity-dependent slowing (resulting in an increase in axonal firing), a reduced incidence of conduction failure related to increased post-spike excitability[55] as well as an increase of the receptive field[56] and in the number of mechanically sensitive C-fibers[56,57]. Rukwied et al. showed that one week after injection in the skin, NGF provoked mechanical, thermal and chemical (but not electrical) peripheral sensitization, which was measured by an increase in axon reflex erythema, compared with vehicle[54].

The aforementioned methods were also used to evaluate the effects of UV-B irradiation in pigs. At 24 and 48 hours after irradiation, Rukwied and colleagues found an increased axon reflex erythema following mechanical and thermal stimulation in the irradiated skin compared with control skin[58]. Hyperalgesia was confirmed by Di Giminiani et al. who observed decreased withdrawal latencies and thresholds upon thermal and mechanical stimulation, respectively, of the irradiated compared to control areas in awake pigs at 24 and 48 hours after UV-B irradiation[59].

Injection of carrageenan in the pig's foot resulted in decreased withdrawal thresholds compared with saline injection[60]. In another study, 1,000 mm^2 lesions were found in the subcutaneous tissue of the pig's back three days after injection of carrageenan. Stretching decreased the size of the lesions and amount of inflammatory cells, but had no effect on pro-resolving inflammatory mediators levels and gene expression associated with inflammation and fibrosis in blood, lesion and muscle tissue[61].

Di Giminiani et al. tested the application of topical capsaicin in pigs. Application of 20 % capsaicin elicited thermal hyperalgesia in small (27 kg) but not large pigs (57 kg), as shown by a decrease in withdrawal latency following thermal stimulation[62].

Irreversible pain models

Peripheral neuritis trauma (PNT)[34,63,64] was developed in 2016 as the first pig model for chronic pain. In this model, three sutures pre-soaked in complete Freund's adjuvant (CFA) were loosely tied around the sciatic nerve. This procedure resulted in increased tactile and mechanical sensitivity from day 7[34,64] and increased pain-related behavior from day 3 compared to baseline and sham operated animals[64]. Motor function was minimally affected from day 3[34,63,64]. The PNT model was compared to the partial and full nerve crush models; in these models decreased withdrawal thresholds were not observed before day 18[64], whereas behavioral scores and motor function were affected already from day 3[64]. At 28 days after PNT surgery, gabapentin and morphine effectively suppressed sensitization for up to 3 hours after administration, while aprepitant failed to suppress sensitization[34].

Disease models. Thirteen papers described specific disease models, including lameness, osteoarthritis, femoral fracture and a genetically modified neurofibromatosis model. Two reviews on spinal cord injury models described pig models, but did not include models in which pain was assessed[30,65]. Another review on neurofibromatosis models mentioned that pain assessment had not yet been carried out in pigs by the time of the review[66] and one of the reviews on spinal cord injury concluded that pain assessment methods are insufficient in pigs[30].

Lameness models

Five studies used a lameness model[67–71]. Lameness was induced by an injection of amphotericin B into the foot and resulted in increased mechanical and thermal sensitivity of the foot[67,69,70]. Furthermore, after lameness induction, the frequency of standing postures decreased and the frequency of lying postures increased; both parameters returned to baseline one week after lameness induction[68]. Several analgesics reduced the sensitization and pain-related behavior for up to three days after lameness induction[67,68], but had no effect after six days[70]. This is most likely because lameness induced by amphotericin B is considered to be resolved after seven days[68]. Lameness and hypersensitivity in the foot were also induced for at least two days using kaolin[71]. Injection of ketoprofen could reduce the foot sensitivity, but not lameness[71].

Other models

Injection of mono-iodoacetate (MIA) in the knees of 27 Yucatan swine resulted in progressive joint damage shown by MRI[72]. Lameness increased in MIA-injected animals from week 2 until week 12 after injection, while kinetic weight-bearing parameters were significantly different between MIA injected and control swine from week 1 after injection[73].

The review on neurofibromatosis mentions that two genetically modified minipig models have been developed to investigate this disease[66]. However, nociceptive assessment was only reported late 2019 where mechanical sensitization was found in female and male Yucatan swine with tumors at 9 months of age, but not afterwards. Thermal sensitization was found in females, and not in males, and only over a longer period of time.[74]

Regional anesthesia had no significant effect on physical and biochemical serum measurements compared to sham during and after surgery on post-operative pain in a femoral fracture model. However, control animals – receiving systemic opioids and non-steroidal anti-inflammatory drugs (NSAIDS) – responded more strongly when approached and required more rescue analgesia, hence providing some evidence for a beneficial effect of regional anesthesia in addition to systemic analgesics.[75]

Pain due to pig production procedures. Pain due to pig production procedures has been relatively thoroughly investigated (30 papers). Sixteen of 30 studies included >100 animals and one study even included 2,888 animals.

Castration

Castration is accompanied by more high-rate high-frequency vocalizations[76–83] and defensive behavior[79,82] than sham castration. Increased pain-related behavior was also reported on the day after castration compared with sham castration[78,80,84–87]. Interestingly, Taylor et al. found that castrates spent more time sitting or standing inactively and less time lying down[78], while Sutherland and colleagues oppositely observed that castrates spent more time lying down without contact[80,86], both compared to sham-castrates. In line with Sutherland et al., another study showed that castrates spent less time walking and running and had less contact with the sow compared with sham castrates[85]. Three groups observed the animals several days after castration and found pain-related behavior 3, 5 and 8 days after castration[85,88,89]. These behaviors rarely occurred in non-castrated animals, apart from lying huddled up[85,89]. The pain-related behavior peaked on the day of castration while scratching and tail wagging peaked on the first day after castration[89]. Only 5 of the 19 studies were blinded[80,84,90–92], which induces a substantial risk of bias given that the assessments might be subjective.

The effectivity of analgesic treatment during castration is not obvious. Generally, anesthetics have been found ineffective[79,80,86,88]. Conflicting results have been reported for most analgesics[82–84,93], except for lidocaine, which consistently reduced pain during castration[81–83].

Tail docking

Ten studies and one review focused on tail docking. Tail docking leads to both more non-specific and specific pain-related behavior (such as tail jamming) during[44,94] and after surgery[92,95,96] compared with handling without tail docking. All studies measuring cortisol showed that cortisol levels increased after tail

docking[92,93,95,97], whereas the effectiveness of the different anesthetic agents was unclear[92,93,95,96]. Acute pain-related behavior during and after tail docking persisted hours to days after the procedure[94,96,97]. Surgical removal of the tail also induced hypersensitivity of the stump in older piglets[98] and changes in gene expression related to inflammatory and neuropathic pain and wound healing[99] for at least 4 months.

Other procedures

Other pig production procedures include ear tagging, ear notching, injection of transponders and teeth clipping. All studies converge to prove that these procedures are more stressful compared to handling the animal without performing the procedures[44,94,100]. Noonan et al. found that each of these procedures led to different pain-related behavior directed at the affected body part[44]. On the other hand, Leslie et al. found that intraperitoneal injection of transponders led to non-specific pain-related behavior (i.e. isolation)[100].

Naturally occurring pain. Four studies investigated pain related to farrowing[101–103]. Behavioral observations were made with a video camera and analyzed using dedicated software, which decreased the risk of bias. Arching of the back, tail flicking and scratching the floor with the foot were increased during farrowing[102]. Back arching is a farrowing-specific behavior, but given that it is also observed during defacation, it may not be pain-related. In a later study, only back arching occurred frequently enough during farrowing to be analyzed[101]. Studies showed no effect of analgesia on this parameter, any other pain-related parameter or any biomarker (cortisol among others)[101,103]. A facial scale with a high sensitivity and reliability has also been developed using farrowing sows[104].

Two studies investigated naturally occurring lameness in lame sows[105] and piglets[106]. In piglets, gait symmetry improved and piglets were more active in the open field after treatment with buprenorphine. All lame piglets were diagnosed post-mortem with arthritis in at least one joint, while the unaffected limbs were without pathology[106]. In sows, mechanical nociception thresholds were significantly higher for healthy limbs compared with lame limbs, for forelimbs compared with hindlimbs and for morning measurements compared with afternoon measurements[105].

Pigs with shoulder ulcers spent more time in active postures and less time lying down compared with pigs without ulcers[107]. However, no differences in behavior directed at the injured shoulder were observed[107].

Duration and intensity of pain

Thirteen of the models described above could be compared quantitatively using mechanical sensitization as outcome measure. The data was normalized to allow for easy comparison between the models. However, it is important to note that there were differences between studies, including the body parts analysed or the investigational tools used. In all cases, the skin was the organ of interest. Most of the models that could be compared belonged to the surrogate pain model category. Pain model durations and intensities are presented in Fig. 3 and 4.

The mechanical sensitization observed in the inflammatory models was mild and apparently short-lasting compared with nerve damage and surgical models. As such, increased axon reflex erythema and decreased withdrawal thresholds were observed following mechanical stimulation 24 and 48 hours post UV-B irradiation, compared with control untreated skin[58,59]. Mechanical sensitization was also observed by

an increase in axon reflex erythema at 1, 3 and 7 days after injection of NGF[54]. Even though the area of mechanically evoked erythema decreased during the seven days, mechanical activation thresholds were still elevated three weeks after injection of NGF[56]. Decreased withdrawal thresholds were found four hours after injection of carrageenan in the foot of the pig compared with injection of saline[60]. The withdrawal thresholds returned to baseline within 24 hours. A study showed a nonsignificant increase in mechanical sensitivity after application of a capsaicin patch compared with a control group[62](Fig. 3a).

For the surgical models, the studies using incisional models presented the most complete datasets. Fig. 3b shows that SI and SMIR models resulted in a 95% decrease in the mechanical withdrawal threshold compared to intact skin measured up to one week after surgery[51–53]. Withdrawal thresholds had not returned to baseline at that time[51–53]. For full surgery models, mechanical sensitization was not investigated, but behavioral measures were recorded in the same manner as for the incisional models [47,51]. Behavioral indicators were severely affected the first hour after full surgery[47] compared with the incision models. Behavioral indicators decreased with time but remained higher than those recorded for incision models until the end of the study (24 hours after surgery)[47].

In the nerve damage models, mechanical sensitization was investigated for the longest duration after induction and none of the studies reported a return to baseline conditions. On day three, behavioral indicators of pain were increased in the different nerve damage models compared with sham pig; the increase was more prominent for the PNT model and less for the partial and full nerve crush models[64]. Withdrawal thresholds were first measured one week after surgery and were only significantly decreased compared with sham for the PNT model[34,64]. Partial and full nerve crush models showed a later decrease in withdrawal thresholds starting at day 18[64] (Fig. 3c).

Tail resection (surgical removal the tail) has been suggested as a potential chronic pain model for neuropathic pain associated with nerve injury, for example amputation[98]. Mechanical sensitization of the stump was found up to four months after resection, with a longer duration of sensitization for younger piglets (9 vs. 17 weeks)[98] (Fig. 3c).

Lameness induced by injection of amphotericin B into the foot resulted in increased mechanical and thermal sensitivity of the foot[67,69,70] (Fig. 4). Although the pigs were considered to have fully recovered after one week, mechanical withdrawal thresholds were still lower compared with baseline and compared with the non-affected leg[67,69,70]. Mechanical hypersensitivity induced by kaolin was similar in intensity to amphotericin B and lasted for the duration of the study (2 days after injection)[71]. Naturally occurring lameness in sows was accompanied by a smaller degree of mechanical sensitization compared with experimentally induced lameness[105].

Pain assessment in humans and pigs

We performed a systematic search for studies in humans using NGF, UV-B, carrageenan and capsaicin to compare pain assessment methods used in human surrogate pain models with those used in the porcine pain models. Eight studies were found for UV-B, 12 for NGF and 9 for capsaicin. No studies using carrageenan were identified. In Table 3, we compare the assessment methods used in these studies with those used in the porcine surrogate pain models to identify possible overlaps.

Evoked responses. Responses have been evoked in human and pigs using electrical, mechanical and thermal stimuli (Table 3). Often, methodologies differ between studies, eg. the investigated body part or the investigational tool. But even when the same pain assessment methodologies are used, different neural substrates may be investigated. In particular, the withdrawal response to pressure or heat in the pig does not necessarily require any cognitive processing[108], while pushing a button in response to pain does. In addition, a human is able not only to indicate pain but also to rate it to quantify its perceived intensity and describe the qualitative aspects of the pain sensation, which an animal of course cannot do. Instead, ethograms have been used in animals as a tool to interpret the withdrawal response to pressure and heat stimuli[59,62]. A body of research in pigs and humans has also applied the same methodology and investigated the same neural substrate, namely peripheral nerve fibers[54,56–58,109,110]. Peripheral nerve activity may be evoked by electrical, mechanical, thermal or chemical stimuli and assessed using, e.g., microneurography or erythema.

Non-evoked responses. Assessment of non-evoked pain in humans is typically carried out by using questionnaires[111–119] and pain ratings on a numerical or visual-analogue scale[111,114,116,118,120,121]. In pigs, observation of pain-related behavior[34,46–48,51,63,64], motor function score[34,46,53,64], grimace scale[90,104,122], open-field test[63] or food consumption[46] have been used. The results of behavioral observations in pigs are often contradictory as previously discussed in the case of castration. Several factors might explain these conficting results: only 5 of 19 pig production studies were blinded and different variable scoring schemes were used across studies; in addition, the presence of the investigator in the room and differences in the interpretation of the animal behavior might have affected the results[5–7].

Physiological responses. Peripheral neurophysiology and skin biopsy have been used both in pig and human surrogate pain models[56,57,64,109]. However, pig surrogate pain studies have not assessed the central nervous system using electrophysiology (Table 3). Two-channel electroencephalogram (EEG) monitoring in castrated piglets showed that the power in all frequency bands and the total power dropped after castration in piglets that had not received lidocaine[123]. The advantage of using EEG in pigs is that the findings can be easily compared with human EEG findings. Additionally, electrodes can be implanted in the pig brain to acquire more detailed information than the data that can be obtained from human studies.

Discussion

Our aim was to compare the currently available pig models within the field of pain research. We used a broad search string and also searched the reference lists of the included literature to provide a comprehensive overview of the current status of this field of research. Twenty-three pain models were identified that induced pain or sensitization lasting from hours to months, although it was not possible to evaluate the exact duration of mechanical sensitization for all the models, given that the majority of the models did not return to baseline by the end of the study. We also found a near to complete overlap of assessment methodologies between human and pig models within the area of peripheral neurophysiology.

What calls for usage of porcine pain models? A broad overlap exists between animal and human models of inflammatory pain. Inflammatory pain models are short-lasting and primarily cause acute activation of nociceptors and relatively mild peripheral sensitization compared with surgical and neuropathic pain models. The axon reflex erythema and activation of particular subclasses of nociceptors might be seen as advantageous in the pig model as these manifestations overlap completely with human responses. However, the traditional measures of nociceptive reflexes or withdrawal thresholds would be clinically

relevant in the case of inflammatory pain[5]. By contrast, neuropathic pain in humans is characterized by a disconnection between severity of neuropathy and clinical pain level and the relationship between structural damage and painful symptoms remains unclear[124]. On the other hand, animal models using nerve damage models have the advantage of producing very robust hypersensitivity[5,17,18]. The PNT model in the pig, which combines ligation and inflammation[34,64], provides a better clinical picture than the traditional nerve damage models[19], because it has both an inflammatory and mechanical aspect[34,63,64]. Moreover, this model results in pain-related behavior, hyperalgesic and allodynic responses while normal locomotion is maintained[34,63,64]. Although the interindividual variability of the pain phenotype after the experimental lesion might be regarded as a drawback when compared with the rodent models, it might also account for the similar variability seen in patients. Moreover, the animals with a less pronounced pain phenotype might provide highly valuable information as a non-painful neuropathy control group. Similarly, sensitized and non-sensitized animals were found among genetically modified neurofibromatose minipigs, in which sensitization was not always related to tumor development[125]. Combined with the fact that pigs respond similarly to humans to analgesics[34,35], the similarities between pig and humans pain manifestations in the PNT and neurofibromatose models clearly warrant a role for the pig in translational pain research.

Pig production procedures and naturally occurring pain can potentially provide valuable porcine pain models. For instance, tail docking has been suggested as a model for stump pain[98]. Tail resection leads to long lasting changes in gene expression in the caudal dorsal root ganglia[99] and a chronic decrease in pressure withdrawal responses of approximately 30%[98]. In terms of intensity, this response is believed to recapitulate what is commonly observed in patients[71,123]. Shoulder ulcers are also commonly observed in lactating pigs kept in a farrowing crate[107]. Further, pigs can also naturally develop arthritis[42,106]. Compared to acute models using inflammatory agents, the natural disease progression includes aspects of ageing and degeneration[42], which provide a more complete clinical representation of the disease in humans[72]. Ethically, the naturally occurring pain conditions are obviously less problematic than other pain models, as pigs are potentially relieved from pain (refinement method). Despite being prohibited in the European Union (council directive 2008/120/EC), tail docking is still a common practice[126]. Given that most pigs undergo tail docking, this model presents the advantage that the animals would not need to undergo additional pain induction, thus reducing the burden of animal studies.

More animal models of chronic pain, mimicking human and clinical pain should still be developed. The pig could be a well-suited species owing to its homology to humans; in particular regarding the nervous system, the skin and the genetic traits[27–30]. On the other hand, many useful models of pain have been developed in rodents, resulting in the identification of several pain mechanisms, such as spinal long term potentiation[14,15]. However, the possibilities to confirm that these mechanisms also exist in humans are limited[127–129]. We therefore suggest to complement rodent models with experimental pig models that hold the promise to reflect human conditions more closely. Based on the results from this systematic search, the pig seems particularly suitable for various purposes. As mentioned above, neuropathic pain models seem to reflect the clinical picture better in pigs than in rodents[34,63,64]. Naturally occurring diseases linked to long-term conditions and degeneration[42,106,107] are more representative of human condition in pigs than in rodents due to the pig's lifespan and bodyweight. The size of the pig also allows for assessment methods more similar to those used in humans, for example EEG[123,130]. The similarities in the peripheral and central nervous system between pigs and humans[27,28,129] also make it possible to more directly compare findings between pig and human studies. Lastly, pharmacokinetic studies are often performed in pigs owing to similarities in permeability and metabolism with humans[45,49]. Given also the great sequence homology

between the two species[27], it is not surprising that analgesic studies in pigs yield similar results to those in humans[34,35].

However, there are also drawbacks to using the pig as a model. Most pig studies use piglets (the pig weighs >100 kg at sexual maturity), while human studies usually involve adults. Additionally, the high growth rate of domestic pigs makes them difficult to use in long-term studies; therefore, various miniature breeds have been developed for biomedical research purposes[45]. However, this adds to the cost of pig research, which is already more expensive than research using rodents. In spite of these drawbacks, we believe that the pig can have an important role in translational pain research.

Duration and intensity of mechanical sensitization in different pain models. Fourteen porcine pain models were compared in terms of intensity and duration. In general, the surgical and nerve damage models had the highest intensity, while the amputation and inflammatory models had the lowest intensity (Fig. 3). Lameness was induced by injection of an inflammatory agent and had a higher intensity than the other inflammatory models. Interestingly, sensitization in naturally occurring lameness was less intense by a factor of 5 compared with inflammatory models (Fig. 4). Of note, the naturally lame sows were housed together, monitored using a remote-controlled algometer and compared with a healthy control group[105], which could have led to lower mechanical nociceptive thresholds and greater variation. Furthermore, experimental lameness was typically investigated on the days were the greatest sensitization was expected[69,70]. Also, the amount of sensitization one week after tail amputation was only half that of the surgical incision models (Fig. 3). As with rodents[18], it could be that pain models in pigs are optimized to consistently achieve the same type of sensitization in every animal. While this means that very few animals are wasted, it also means that the animal model deviates from the clinically relevant profile, which in turn may result in difficulties of translating the results to the clinic.

This quantitative comparison can also be extended to human inflammatory models. This comparison has been made directly for the NGF models, and similarities between humans and pigs were found[55,56]. UV-B radiation (1 J/cm^2) yielded a lower withdrawal threshold in pigs[59] than the pain threshold observed in humans after radiation (maximum dose of 0.3 J/cm^2)[119]. The capsaicin model did not decrease the mechanical withdrawal threshold in pigs[62], while mechanical and pinprick hyperalgesia have been reported in human models[121,131]. While there is a good consistency in peripheral electrophysiology between pigs and humans, the withdrawal thresholds in pigs seem consistently higher than the pain thresholds in humans. We believe that these observations point towards the need for more objective measures in both species as a way to facilitate the translation of results between species.

The duration could not be determined for all models as mechanical sensitivity returned to baseline in only two studies[60,62]. Mechanical sensitization could be expected to also return to baseline for surgical models, but the purpose of these studies was to test analgesics during the time the animals were sensitized[47,52,53]. This underscores the more general problem that the purpose of pain studies is usually not to investigate the duration of the pain as it is unethical to let the animals suffer for a longer amount of time than needed.

Another limitation of this comparison is that the included models were predominantly surrogate pain models (10 models) while only two disease models, one naturally occurring pain model and one pig production procedure model, were included. This is most likely due to the outcome measure selected to extract the data, i.e. mechanical sensitivity. Mechanical sensitivity is a very common outcome measure in

pain research, but not necessarily a relevant measure in pig production. In studies related to pig production, animal behavior was assessed more often than other outcomes, but in a non-standardized way, which made the articles unsuitable for data extraction and analysis in this review[38].

Why the choice of better assessment methods may be the key to a successful pig-to-human translation. An advantage of pain assessment in pigs is that scaling effects between human and pig studies can be avoided, which is not possible with rodents. In pigs, evoked responses can be assessed using methods similar to those used in humans[132]. However, even when the same methods are used, a different neural substrate can be investigated (e.g. pain rating in humans and withdrawal in animals)[7,27]. Nevertheless, the use of nerve fiber activation and erythema as outcomes has created an overlap between pig and human experiments[54,56–58,109,110].

Many pig studies, in particular in the pig production field, have assessed non-evoked pain behavior. Such assessments are relevant for translational research, as these outcome measures are used in clinical research[5,7]. To some extent, such assessments reflect a complex behavior involving cortical structures [27]. However, the results are variable between studies and seemingly unreliable. Blinding is an obvious necessity when pain-related behavior in animals is evaluated. In many studies, a set of behavioral responses were predetermined to be pain-related, while literature suggests that various behaviors can be expected. This may bias the results towards finding increased pain-related behavior in animals receiving a painful intervention when the observer is not blinded. Consequently, all studies using a specific pain-related behavior (which was different between studies) found that tail docked or castrated animals showed more of this behavior compared with sham-treated animals[44,85,89,100], except one study which found no difference in the percentage of pain-related behavior[95]. Overall, behavioral responses after pig production procedures are conflicting; walking more[89] and less[85,90], lying down more[80,86,95] and less[78,94] and sitting more[78] and less[85] have all been reported as a sign of the animal being in pain. These results might indicate that the level of non-evoked pain is too low to be detected in a robust way or highlight the need for more objective assessments of pain-related behavior in pigs, as there is no standardized ethogram, and no consistency regarding the age of the piglets or the sampling methodology. Many of these studies also used automated analysis of vocal recordings. This analysis has provided consistent results – during a painful procedure (castration, tail docking or ear notching) a higher percentage of high-frequency calls has been recorded[76,78,78–80,83,86,94,95] – whereas quantification of other behaviors using an ethogram has failed. Therefore, we suggest to also record animal behavior by means of video[101,102] and to develop automated software to analyze behaviors that consistently have been observed after painful interventions. This methodology has the potential to resolve the issues of blinding, experimenter bias, the presence of an observer in the room, scoring scheme and interpretation, thereby eliminating multiple sources of bias[7,38,42]. Recently, efforts have been made to validate behavioral assessment methods in pigs, such as the grimace scale[91,104], which is a step in the right direction. Such efforts will allow the use of a measure that is immediate, sensitive and in certain cases specific[133] even when the pig is unattended, which we expect to be particularly valuable when used in an objective way.

Numerous studies have attempted to find other methods to objectively investigate non-evoked pain: navigation time through a handling chute[134], eye temperature, latency to move, locomotor activity[87], rubbing[107], open-field movements[63], facial expressions[90,104,122] and biomarkers such as cortisol concentration[80,83,84,86,87]. However, cortisol is a hormone released in response to stress and, although pain is a very potent stressor, an increase in the cortisol level alone cannot distinguish between pain and stress. The same question of specificity could be raised for all of these measures**.** Nevertheless, these parameters

could still be used in combination with other assessment methods to give more insight into a painful condition.

During the last decade, novel methods have been developed for evaluating spontaneous activity in rodents before and after induction of a pain including dynamic weight bearing, place preference on plate with temperature gradient, catwalk and facial assessments[135]. These methods contribute to our understanding of rodent behavior and have been used to evaluate therapies to alleviate pain[136,137]; similar methods have been used in pigs[73,90,104,122]. Motivational tasks are also available for pigs, such as judgement bias, discrimination, gambling tasks[138] and the marshmallow test[139]. The T-maze task was used in the development of the neurofibromatosis model to identify learning deficits[66,125], but such tests have not yet been used for pain research in pigs. Motivational tests would add a different insight into pain-related behavior given that they investigate higher brain functions[27]. However, these tests remain unspecific tests that are influenced by many other factors aside from pain.

Electrophysiology has been a particularly useful tool to objectively and specifically assess the peripheral pain system in pigs[55–58]. However, a major knowledge gap still exists when it comes to central pain pathways. Only two studies have obtained central recordings using the bispectral index for EEG monitoring[123,130]. While EEG measurements are a valuable way to bridge the gap between human and porcine pain research, we also believe that pigs offer the prospect of more invasive brain measurements that may provide a deeper understanding of central pain processing. Thus, pain research in pigs provides an excellent opportunity to complement human pain research and overcome its limitation (such as limited quality of neuronal recordings and availability of neuronal tissue in humans), by using a set of common stimulation and test paradigms (Fig. 5).

Conclusions

Based on the literature, we identified 23 animal models related to pain. Fourteen of these pain models induced a mechanical sensitization lasting from hours to months and with intensities ranging from nonsubstantial to maximal mechanical sensitization. The pig model seems to be particularly relevant for pain research with regard to naturally occurring diseases (e.g. arthritis) and pharmacokinetic studies. Other advantages of the pig include a neuropathic pain model that separates between neuropathy, pain and motor dysfunction and a lack of scaling problems. Given the physiological similarities between pigs an humans, assessment methodologies available in pigs and based on peripheral neurophysiology and erythema, can be used in both species. We conclude that these aspects warrant a role for the pig model in translational pain research. We suggest that studies on pain in porcine models use evoked, non-evoked and physiological assessments as outcome measures. Much is to be gained by further refining pain assessment in pigs, in particular by objectifying behavioral assessment and by exploiting the similarity of central nervous system circuits between humans and pigs. We expect that the use of pig models will provide new information about clinically relevant pain mechanisms.

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Author contributions

S Meijs conceived the original work, carried out data acquisition, analysis, interpretation and wrote the draft and revisions of the manuscript. M Schmelz and S Meijlin revised the manuscript according to their expertise. W Jensen contributed to conceiving the work, supported data interpretation and drafting and revision of the manuscript.

Conflict of interest

The authors declare no competing interests.

References

1. Breivik, H., Eisenberg, E. & O'Brien, T. The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. *BMC Public Health* **13**, 1229 (2013).
2. Institute of Medicine (US) Committee on Advancing Pain Research, Care, and Education. *Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research.* (National Academies Press (US)).
3. Reid, K. J. *et al.* Epidemiology of chronic non-cancer pain in Europe: narrative review of prevalence, pain treatments and pain impact. *Curr. Med. Res. Opin.* **27**, 449–462 (2011).
4. Hill, R. NK1 (substance P) receptor antagonists – why are they not analgesic in humans? *Trends Pharmacol. Sci.* **21**, 244–246 (2000).
5. Mogil, J. S. Animal models of pain: progress and challenges. *Nat. Rev. Neurosci.* **10**, 283–294 (2009).
6. Burma, N. E., Leduc-Pessah, H., Fan, C. Y. & Trang, T. Animal models of chronic pain: Advances and challenges for clinical translation: Animal Models of Chronic Pain. *J. Neurosci. Res.* **95**, 1242–1256 (2017).
7. Percie du Sert, N. & Rice, A. S. C. Improving the translation of analgesic drugs to the clinic: animal models of neuropathic pain: Improving models of neuropathic pain. *Br. J. Pharmacol.* **171**, 2951–2963 (2014).
8. Taxonomy Working Group. *Classification of chronic pain*. (IASP Press, 2011).
9. Melzack, R. The McGill Pain Questionnaire: major properties and scoring methods. *Pain* **1**, 277–299 (1975).

10. Rose, J. D. & Woodbury, C. J. Animal Models of Nociception and Pain. in *Sourcebook of models for biomedical research* (ed. Conn, P. M.) 333–340 (Humana Press, 2008).
11. Haroutounian, S. *et al.* How central is central poststroke pain? The role of afferent input in poststroke neuropathic pain: a prospective, open-label pilot study. *PAIN* **159**, 1317–1324 (2018).
12. Le Bars, D., Dickenson, A. H. & Besson, J. M. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. *Pain* **6**, 283–304 (1979).
13. Woolf, C. J. Evidence for a central component of post-injury pain hypersensitivity. *Nature* **306**, 686–688 (1983).
14. Liu, X.-G. & Sandkühler, J. Characterization of Long-Term Potentiation of C-Fiber–Evoked Potentials in Spinal Dorsal Horn of Adult Rat: Essential Role of NK1 and NK2 Receptors. *J. Neurophysiol.* **78**, 1973–1982 (1997).
15. Sandkühler, J. & Liu, X. Induction of long-term potentiation at spinal synapses by noxious stimulation or nerve injury: LTP in spinal cord induced by noxious stimulation. *Eur. J. Neurosci.* **10**, 2476–2480 (1998).
16. Sandkühler, J. Models and Mechanisms of Hyperalgesia and Allodynia. *Physiol. Rev.* **89**, 707–758 (2009).
17. Campbell, J. N. & Meyer, R. A. Mechanisms of Neuropathic Pain. *Neuron* **52**, 77–92 (2006).
18. Decosterd, I. & Woolf, C. J. Spared nerve injury: an animal model of persistent peripheral neuropathic pain: *Pain* **87**, 149–158 (2000).
19. Jaggi, A. S., Jain, V. & Singh, N. Animal models of neuropathic pain: Animal models of neuropathic pain. *Fundam. Clin. Pharmacol.* **25**, 1–28 (2011).
20. Casals-Díaz, L., Vivó, M. & Navarro, X. Nociceptive responses and spinal plastic changes of afferent C-fibers in three neuropathic pain models induced by sciatic nerve injury in the rat. *Exp. Neurol.* **217**, 84–95 (2009).

21. Shields, S. D. *et al.* Insensitivity to Pain upon Adult-Onset Deletion of Nav1.7 or Its Blockade with Selective Inhibitors. *J. Neurosci.* **38**, 10180–10201 (2018).

22. Tappe-Theodor, A., King, T. & Morgan, M. M. Pros and Cons of Clinically Relevant Methods to Assess Pain in Rodents. *Neurosci. Biobehav. Rev.* **100**, 335–343 (2019).

23. Dostrovsky, J. & Craig, A. Ascending projection systems. in *Wall & Melzack's Textbook of Pain.* 182–197 (Elsevier Health Sciences, 2013).

24. McIntyre, P. *et al.* Pharmacological differences between the human and rat vanilloid receptor 1 (VR1). *Br. J. Pharmacol.* **132**, 1084–1094 (2001).

25. Whiteside, G. T., Adedoyin, A. & Leventhal, L. Predictive validity of animal pain models? A comparison of the pharmacokinetic–pharmacodynamic relationship for pain drugs in rats and humans. *Neuropharmacology* **54**, 767–775 (2008).

26. Henze, D. A. & Urban, M. O. Chapter 17 Large Animal Models for Pain Therapeutic Development. 17.

27. Cobianchi, L. *et al.* Pain assessment in animal models: do we need further studies? *J. Pain Res.* 227 (2014) doi:10.2147/JPR.S59161.

28. Sauleau, P., Lapouble, E., Val-Laillet, D. & Malbert, C.-H. The pig model in brain imaging and neurosurgery. *animal* **3**, 1138–1151 (2009).

29. Schmidt, V. Comparative anatomy of the pig brain - An integrative magnetic resonance imaging (MRI) study of the porcine brain with special emphasis on the external morphology of the cerebral cortex. (2015).

30. Schomberg, D. T. *et al.* Translational Relevance of Swine Models of Spinal Cord Injury. *J. Neurotrauma* **34**, 541–551 (2017).

31. Lynn, B., Faulstroh, K. & Pierau, F.-K. The Classification and Properties of Nociceptive Afferent Units from the Skin of the Anaesthetized Pig. *Eur. J. Neurosci.* **7**, 431–437 (1995).

32. Ohta, T., Komatsu, R., Imagawa, T., Otsuguro, K. & Ito, S. Molecular cloning, functional characterization of the porcine transient receptor potential V1 (pTRPV1) and pharmacological comparison with endogenous pTRPV1. *Biochem. Pharmacol.* **71**, 173–187 (2005).

33. Dusch, M. *et al.* Comparison of electrically induced flare response patterns in human and pig skin. *Inflamm. Res.* **58**, 639–648 (2009).

34. Castel, D., Sabbag, I., Brenner, O. & Meilin, S. Peripheral Neuritis Trauma in Pigs: A Neuropathic Pain Model. *J. Pain* **17**, 36–49 (2016).

35. Ottoboni, T. *et al.* Mechanism of action of HTX-011: a novel, extended-release, dual-acting local anesthetic formulation for postoperative pain. *Reg. Anesth. Pain Med.* **45**, 117–123 (2020).

36. Viscusi, E. *et al.* HTX-011 reduced pain intensity and opioid consumption versus bupivacaine HCl in herniorrhaphy: results from the phase 3 EPOCH 2 study. *Hernia* **23**, 1071–1080 (2019).

37. Sutherland, M. Welfare implications of invasive piglet husbandry procedures, methods of alleviation and alternatives: a review. *N. Z. Vet. J.* **63**, 52–57 (2015).

38. Dzikamunhenga, R. S. *et al.* Pain management in the neonatal piglet during routine management procedures. Part 1: a systematic review of randomized and non-randomized intervention studies. *Anim. Health Res. Rev.* **15**, 14–38 (2014).

39. O'Connor, A. *et al.* Pain management in the neonatal piglet during routine management procedures. Part 2:Grading the quality of evidence and the strength of recommendations. *Anim. Health Res. Rev.* **15**, 39–62 (2014).

40. O'Connor, A. *et al.* Review: Assessment of completeness of reporting in intervention studies using livestock: an example from pain mitigation interventions in neonatal piglets. *animal* **10**, 660–670 (2016).

41. Ison, S. H., Clutton, R. E., Di Giminiani, P. & Rutherford, K. M. D. A Review of Pain Assessment in Pigs. *Front. Vet. Sci.* **3**, (2016).

42. Herskin, M. S. & Di Giminiani, P. Pain in pigs. in *Advances in Pig Welfare* 325–355 (Elsevier, 2018). doi:10.1016/B978-0-08-101012-9.00011-3.

43. Ison, S. H., Clutton, R. E., Di Giminiani, P. & Rutherford, K. M. D. A Review of Pain Assessment in Pigs. *Front. Vet. Sci.* **3**, (2016).

44. Noonan, G. J., Rand, J. S., Priest, J., Ainscow, J. & Blackshaw, J. K. Behavioural observations of piglets undergoing tail docking, teeth clipping and ear notching. *Appl. Anim. Behav. Sci.* **39**, 203–213 (1994).

45. Swindle, M. M., Makin, A., Herron, A. J., Clubb, F. J. & Frazier, K. S. Swine as Models in Biomedical Research and Toxicology Testing. *Vet. Pathol.* **49**, 344–356 (2012).

46. Harvey-Clark, C. J., Gilespie, K. & Riggs, K. W. Transdermal fentanyl compared with parenteral buprenorphine in post-surgical pain in swine: a case study. *Lab. Anim.* **34**, 386–398 (2000).

47. Reyes, L. Observer-blinded comparison of two nonopioid analgesics for postoperative pain in piglets. *Pharmacol. Biochem. Behav.* **73**, 521–528 (2002).

48. Malavasi, L. M., Nyman, G., Augustsson, H., Jacobson, M. & Jensen-Waern, M. Effects of epidural morphine and transdermal fentanyl analgesia on physiology and behaviour after abdominal surgery in pigs. *Lab. Anim.* **40**, 16–27 (2006).

49. Lykkegaard, K., Lykkesfeldt, J., Lauritzen, B. & Svendsen, O. Morphine reduces spinal c-fos expression dose-dependently during experimental laparotomy in pigs: A combined pharmacokinetic and surgical study. *Res. Vet. Sci.* **84**, 457–464 (2008).

50. Lykkegaard, K., Lauritzen, B., Tessem, L., Weikop, P. & Svendsen, O. Local anaesthetics attenuates spinal nociception and HPA-axis activation during experimental laparotomy in pigs. *Res. Vet. Sci.* **79**, 245–251 (2005).

51. Castel, D., Willentz, E., Doron, O., Brenner, O. & Meilin, S. Characterization of a porcine model of post-operative pain: A pig model of incisional pain. *Eur. J. Pain* **18**, 496–505 (2014).

52. Castel, D., Sabbag, I. & Meilin, S. The effect of local/topical analgesics on incisional pain in a pig model. *J. Pain Res.* **Volume 10**, 2169–2175 (2017).

53. Wilsey, J. T. & Block, J. Sustained analgesic effect of clonidine co-polymer depot in a porcine incisional pain model. *J. Pain Res.* **Volume 11**, 693–701 (2018).

54. Rukwied, R. *et al.* Nerve growth factor-evoked nociceptor sensitization in pig skin in vivo. *J. Neurosci. Res.* NA-NA (2010) doi:10.1002/jnr.22351.

55. Obreja, O. *et al.* NGF enhances electrically induced pain, but not axon reflex sweating: *Pain* **152**, 1856–1863 (2011).

56. Hirth, M. *et al.* Nerve growth factor induces sensitization of nociceptors without evidence for increased intraepidermal nerve fiber density: *Pain* **154**, 2500–2511 (2013).

57. Petersson, M. E. *et al.* Differential Axonal Conduction Patterns of Mechano-Sensitive and Mechano-Insensitive Nociceptors – A Combined Experimental and Modelling Study. *PLoS ONE* **9**, e103556 (2014).

58. Rukwied, R., Dusch, M., Schley, M., Forsch, E. & Schmelz, M. Nociceptor sensitization to mechanical and thermal stimuli in pig skin in vivo. *Eur. J. Pain* **12**, 242–250 (2008).

59. Di Giminiani, P., Petersen, L. J. & Herskin, M. S. Characterization of nociceptive behavioural responses in the awake pig following UV-B-induced inflammation: UV-B induced hyperalgesia in the pig. *Eur. J. Pain* **18**, 20–28 (2014).

60. Sandercock, D. A. *et al.* Development of a mechanical stimulator and force measurement system for the assessment of nociceptive thresholds in pigs. *J. Neurosci. Methods* **182**, 64–70 (2009).

61. Vergara, D. M. *et al.* Establishment of a Novel Porcine Model to Study the Impact of Active Stretching on a Local Carrageenan-Induced Inflammation. *Am. J. Phys. Med. Rehabil.* **99**, 1012–1019 (2020).

62. Di Giminiani, P., Petersen, L. J. & Herskin, M. S. Capsaicin-induced neurogenic inflammation in pig skin: A behavioural study. *Res. Vet. Sci.* **96**, 447–453 (2014).

63. Castel, D., Sabbag, I., Nasaev, E., Peng, S. & Meilin, S. Open field and a behavior score in PNT model for neuropathic pain in pigs. *J. Pain Res.* **Volume 11**, 2279–2293 (2018).

64. Rice, F. L. *et al.* Human-like cutaneous neuropathologies associated with a porcine model of peripheral neuritis: A translational platform for neuropathic pain. *Neurobiol. Pain* **5**, 100021 (2019).

65. Goel, S. A., Varghese, V. & Demir, T. Animal models of spinal injury for studying back pain and SCI. *J. Clin. Orthop. Trauma* **11**, 816–821 (2020).

66. Bellampalli, S. S. & Khanna, R. Towards a neurobiological understanding of pain in neurofibromatosis type 1: mechanisms and implications for treatment. *Pain* **160**, 1007–1018 (2019).

67. Pairis-Garcia, M. *et al.* Measuring the efficacy of flunixin meglumine and meloxicam for lame sows using nociceptive threshold tests. *Anim. Welf.* **23**, 219–229 (2014).

68. Pairis-Garcia, M. *et al.* Behavioural evaluation of analgesic efficacy for pain mitigation in lame sows. *Anim. Welf.* **24**, 93–99 (2015).

69. Mohling, C. M. *et al.* Evaluation of mechanical and thermal nociception as objective tools to measure painful and nonpainful lameness phases in multiparous sows. *J. Anim. Sci.* **92**, 3073–3081 (2014).

70. Tapper, K. R. *et al.* Pressure algometry and thermal sensitivity for assessing pain sensitivity and effects of flunixin meglumine and sodium salicylate in a transient lameness model in sows. *Livest. Sci.* **157**, 245–253 (2013).

71. Fosse, T. K. *et al.* Ketoprofen in piglets: enantioselective pharmacokinetics, pharmacodynamics and PK/PD modelling: Pharmacokinetics and pharmacodynamics of ketoprofen in piglets. *J. Vet. Pharmacol. Ther.* **34**, 338–349 (2011).

72. Unger, M. D. *et al.* Clinical magnetic resonance-enabled characterization of mono-iodoacetate-induced osteoarthritis in a large animal species. *PLOS ONE* **13**, e0201673 (2018).

73. LaVallee, K. T. *et al.* Quantitation of Gait and Stance Alterations Due to Monosodium Iodoacetate–induced Knee Osteoarthritis in Yucatan Swine. *Comp. Med.* **70**, 248–257 (2020).

74. Khanna, R. *et al.* Assessment of nociception and related quality-of-life measures in a porcine model of neurofibromatosis type 1. *Pain* **160**, 2473–2486 (2019).

75. Royal, J. M. *et al.* Assessment of Postoperative Analgesia after Application of Ultrasound-Guided Regional Anesthesia for Surgery in a Swine Femoral Fracture Model. *J. Am. Assoc. Lab. Anim. Sci.* **52**, 12 (2013).

76. Weary, D. M., Braithwaite, L. A. & Fraser, D. Vocal response to pain in piglets. *Appl. Anim. Behav. Sci.* **56**, 161–172 (1998).

77. Taylor, A. A. & Weary, D. M. Vocal responses of piglets to castration: identifying procedural sources of pain. *Appl. Anim. Behav. Sci.* **70**, 17–26 (2000).

78. Taylor, A. A., Weary, D. M., Lessard, M. & Braithwaite, L. Behavioural responses of piglets to castration: the effect of piglet age. *Appl. Anim. Behav. Sci.* **73**, 35–43 (2001).

79. Leidig, M. S., Hertrampf, B., Failing, K., Schumann, A. & Reiner, G. Pain and discomfort in male piglets during surgical castration with and without local anaesthesia as determined by vocalisation and defence behaviour. *Appl. Anim. Behav. Sci.* **116**, 174–178 (2009).

80. Sutherland, M. A., Davis, B. L., Brooks, T. A. & McGlone, J. J. Physiology and behavior of pigs before and after castration: effects of two topical anesthetics. *animal* **4**, 2071–2079 (2010).

81. Marx, G., Horn, T., Thielebein, J., Knubel, B. & von Borell, E. Analysis of pain-related vocalization in young pigs. *J. Sound Vib.* **266**, 687–698 (2003).

82. Hansson, M., Lundeheim, N., Nyman, G. & Johansson, G. Effect of local anaesthesia and/or analgesia on pain responses induced by piglet castration. *Acta Vet. Scand.* **53**, 34 (2011).

83. Kluivers-Poodt, M. *et al.* Effects of a local anaesthetic and NSAID in castration of piglets, on the acute pain responses, growth and mortality. *Animal* **6**, 1469–1475 (2012).

84. Keita, A., Pagot, E., Prunier, A. & Guidarini, C. Pre–emptive meloxicam for postoperative analgesia in piglets undergoing surgical castration. *Vet. Anaesth. Analg.* **37**, 367–374 (2010).

85. Llamas Moya, S., Boyle, L. A., Lynch, P. B. & Arkins, S. Effect of surgical castration on the behavioural and acute phase responses of 5-day-old piglets. *Appl. Anim. Behav. Sci.* **111**, 133–145 (2008).

86. Sutherland, M. A., Davis, B. L., Brooks, T. A. & Coetzee, J. F. The physiological and behavioral response of pigs castrated with and without anesthesia or analgesia1. *J. Anim. Sci.* **90**, 2211–2221 (2012).

87. Lonardi, C., Scollo, A., Normando, S., Brscic, M. & Gottardo, F. Can novel methods be useful for pain assessment of castrated piglets? *Animal* **9**, 871–877 (2015).

88. Van Beirendonck, S., Driessen, B., Verbeke, G. & Geers, R. Behavior of piglets after castration with or without carbon dioxide anesthesia1. *J. Anim. Sci.* **89**, 3310–3317 (2011).

89. Hay, M., Vulin, A., Génin, S., Sales, P. & Prunier, A. Assessment of pain induced by castration in piglets: behavioral and physiological responses over the subsequent 5 days. *Appl. Anim. Behav. Sci.* **82**, 201–218 (2003).

90. Di Giminiani, P. *et al.* The Assessment of Facial Expressions in Piglets Undergoing Tail Docking and Castration: Toward the Development of the Piglet Grimace Scale. *Front. Vet. Sci.* **3**, (2016).

91. Luna, S. P. L. *et al.* Validation of the UNESP-Botucatu pig composite acute pain scale (UPAPS). *PLOS ONE* **15**, e0233552 (2020).

92. Tenbergen, R., Friendship, R. & Haley, D. Investigation of the use of meloxicam for reducing pain associated with castration and tail docking and improving performance in piglets. *J. Swine Health Prod.* **22**, 7 (2014).

93. Bates, J. L. *et al.* Impact of Transmammary-Delivered Meloxicam on Biomarkers of Pain and Distress in Piglets after Castration and Tail Docking. *PLoS ONE* **9**, e113678 (2014).

94. Torrey, S., Devillers, N., Lessard, M., Farmer, C. & Widowski, T. Effect of age on the behavioral and physiological responses of piglets to tail docking and ear notching1. *J. Anim. Sci.* **87**, 1778–1786 (2009).

95. Sutherland, M. A., Davis, B. L. & McGlone, J. J. The effect of local or general anesthesia on the physiology and behavior of tail docked pigs. *Animal* **5**, 1237–1246 (2011).

96. Herskin, M. S., Di Giminiani, P. & Thodberg, K. Effects of administration of a local anaesthetic and/or an NSAID and of docking length on the behaviour of piglets during 5 h after tail docking. *Res. Vet. Sci.* **108**, 60–67 (2016).

97. Sutherland, M. A., Bryer, P. J., Krebs, N. & McGlone, J. J. Tail docking in pigs: acute physiological and behavioural responses. *animal* **2**, 292–297 (2008).

98. Di Giminiani, P. *et al.* Characterization of short- and long-term mechanical sensitisation following surgical tail amputation in pigs. *Sci. Rep.* **7**, 4827 (2017).

99. Sandercock, D. A. *et al.* Transcriptomics Analysis of Porcine Caudal Dorsal Root Ganglia in Tail Amputated Pigs Shows Long-Term Effects on Many Pain-Associated Genes. *Front. Vet. Sci.* **6**, (2019).

100. Leslie, E., Hernández-Jover, M., Newman, R. & Holyoake, P. Assessment of acute pain experienced by piglets from ear tagging, ear notching and intraperitoneal injectable transponders. *Appl. Anim. Behav. Sci.* **127**, 86–95 (2010).

101. Ison, S. H., Jarvis, S., Hall, S. A., Ashworth, C. J. & Rutherford, K. M. D. Periparturient Behavior and Physiology: Further Insight Into the Farrowing Process for Primiparous and Multiparous Sows. *Front. Vet. Sci.* **5**, 122 (2018).

102. Ison, S. H., Jarvis, S. & Rutherford, K. M. D. The identification of potential behavioural indicators of pain in periparturient sows. *Res. Vet. Sci.* **109**, 114–120 (2016).

103. Viitasaari, E. *et al.* The effect of ketoprofen on post-partum behaviour in sows. *Appl. Anim. Behav. Sci.* **158**, 16–22 (2014).

104. Navarro, E., Mainau, E. & Manteca, X. Development of a Facial Expression Scale Using Farrowing as a Model of Pain in Sows. *Animals* **10**, 2113 (2020).

105. Nalon, E. *et al.* Mechanical nociception thresholds in lame sows: Evidence of hyperalgesia as measured by two different methods. *Vet. J.* **198**, 386–390 (2013).

106. Meijer, E., van Nes, A., Back, W. & van der Staay, F. J. Clinical effects of buprenorphine on open field behaviour and gait symmetry in healthy and lame weaned piglets. *Vet. J.* **206**, 298–303 (2015).

107. Larsen, T., Kaiser, M. & Herskin, M. S. Does the presence of shoulder ulcers affect the behaviour of sows? *Res. Vet. Sci.* **98**, 19–24 (2015).

108. Schouenborg, J. & Kalliomiki, J. Functional organization of the nociceptive withdrawal reflexes. *Exp. Brain Res.* **83**, 67–78 (1990).

109. Obreja, O. *et al.* Nerve growth factor locally sensitizes nociceptors in human skin: *PAIN* **159**, 416–426 (2018).

110. Rukwied, R., Weinkauf, B., Main, M., Obreja, O. & Schmelz, M. Axonal hyperexcitability after combined NGF sensitization and UV-B inflammation in humans: Axonal hyperexcitability after combined NGF and UV-B. *Eur. J. Pain* **18**, 785–793 (2014).

111. Sørensen, L. B., Gazerani, P. & Graven-Nielsen, T. Nerve growth factor-induced muscle hyperalgesia facilitates ischaemic contraction-evoked pain. *Eur. J. Pain* **23**, 1814–1825 (2019).

112. Schabrun, S. M., Christensen, S. W., Mrachacz-Kersting, N. & Graven-Nielsen, T. Motor Cortex Reorganization and Impaired Function in the Transition to Sustained Muscle Pain. *Cereb. Cortex* **26**, 1878–1890 (2016).

113. Enax-Krumova, E. K., Pohl, S., Westermann, A. & Maier, C. Ipsilateral and contralateral sensory changes in healthy subjects after experimentally induced concomitant sensitization and hypoesthesia. *BMC Neurol.* **17**, (2017).

114. Costa, Y. M. *et al.* Masseter corticomotor excitability is decreased after intramuscular administration of nerve growth factor. *Eur. J. Pain* **23**, 1619–1630 (2019).

115. De Martino, E., Zandalasini, M., Schabrun, S., Petrini, L. & Graven-Nielsen, T. Experimental muscle hyperalgesia modulates sensorimotor cortical excitability, which is partially altered by unaccustomed exercise: *PAIN* **159**, 2493–2502 (2018).

116. Sørensen, L. B., Boudreau, S. A., Gazerani, P. & Graven-Nielsen, T. Enlarged Areas of Pain and Pressure Hypersensitivityby Spatially Distributed Intramuscular Injections ofLow-Dose Nerve Growth Factor. *J. Pain* **20**, 566–576 (2019).

117. Exposto, F., Masuda, M., Castrillon, E. & Svensson, P. Effects of nerve growth factor experimentally-induced craniofacial muscle sensitization on referred pain frequency and number of headache days: A double-blind, randomized placebo-controlled study. *Cephalalgia* **38**, 2006–2016 (2018).
118. Munkholm, T. K. & Arendt-Nielsen, L. The interaction between NGF-induced hyperalgesia and acid-provoked pain in the infrapatellar fat pad and tibialis anterior muscle of healthy volunteers. *Eur. J. Pain* **21**, 474–485 (2017).
119. Lo Vecchio, S. *et al.* Interaction between ultraviolet B-induced cutaneous hyperalgesia and nerve growth factor-induced muscle hyperalgesia. *Eur. J. Pain* **20**, 1058–1069 (2016).
120. Furman, A. J. *et al.* Cerebral peak alpha frequency reflects average pain severity in a human model of sustained, musculoskeletal pain. *J. Neurophysiol.* **122**, 1784–1793 (2019).
121. Liu, M., Max, M. B., Robinovitz, E., Gracely, R. H. & Bennett, G. J. The Human Capsaicin Model of Allodynia and Hyperalgesia: Sources of Variability and Methods for Reduction. **16**, 11 (1998).
122. McLennan, K. M. *et al.* Conceptual and methodological issues relating to pain assessment in mammals: The development and utilisation of pain facial expression scales. *Appl. Anim. Behav. Sci.* **217**, 1–15 (2019).
123. Haga, H. A. & Ranheim, B. Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Vet. Anaesth. Analg.* **32**, 1–9 (2005).
124. Karlsson, P., Hincker, A. M., Jensen, T. S., Freeman, R. & Haroutounian, S. Structural, functional, and symptom relations in painful distal symmetric polyneuropathies: a systematic review. *PAIN* **160**, 286–297 (2019).
125. Khanna, R. *et al. Sex-dependent differences in pain and sleep in a porcine model of Neurofibromatosis type 1: Table 1*. http://biorxiv.org/lookup/doi/10.1101/495358 (2018) doi:10.1101/495358.
126. De Briyne, N., Berg, C., Blaha, T., Palzer, A. & Temple, D. 'Phasing out pig tail docking in the EU - present state, challenges and possibilities'. *Porc. Health Manag.* **4**, 27 (2018).

127. Klein, T., Magerl, W. & Treede, R.-D. Perceptual Correlate of Nociceptive Long-Term Potentiation (LTP) in Humans Shares the Time Course of Early-LTP. *J. Neurophysiol.* **96**, 3551–3555 (2006).

128. Magerl, W., Hansen, N., Treede, R.-D. & Klein, T. The human pain system exhibits higher-order plasticity (metaplasticity). *Neurobiol. Learn. Mem.* **154**, 112–120 (2018).

129. Henrich, F., Magerl, W., Klein, T., Greffrath, W. & Treede, R.-D. Capsaicin-sensitive C- and A-fibre nociceptors control long-term potentiation-like pain amplification in humans. *Brain* **138**, 2505–2520 (2015).

130. Haga, H. A., Tevik, A. & Moerch, H. Electroencephalographic and cardiovascular indicators of nociception during isoflurane anaesthesia in pigs. *Vet. Anaesth. Analg.* **28**, 126–131 (2001).

131. Zheng, Z., Gibson, S. J., Khalil, Z., Helme, R. D. & McMeeken, J. M. Age-related differences in the time course of capsaicin-induced hyperalgesia: *Pain* **85**, 51–58 (2000).

132. Rolke, R. *et al.* Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values: *Pain* **123**, 231–243 (2006).

133. Prunier, A. *et al.* Identifying and monitoring pain in farm animals: a review. *animal* **7**, 998–1010 (2013).

134. Bilsborrow, K., Seddon, Y. M., Brown, J., Waldner, C. & Stookey, J. M. An investigation of a novel behavioural test to assess pain in piglets following castration. *Can. J. Anim. Sci.* **96**, 376–385 (2016).

135. Deuis, J. R., Dvorakova, L. S. & Vetter, I. Methods Used to Evaluate Pain Behaviors in Rodents. *Front. Mol. Neurosci.* **10**, (2017).

136. Matsumiya, L. C. *et al.* Using the Mouse Grimace Scale to Reevaluate the Efficacy of Postoperative Analgesics in Laboratory Mice. *J. Am. Assoc. Lab. Anim. Sci.* **51**, 8 (2012).

137. Ängeby Möller, K. *et al.* Gait analysis and weight bearing in pre-clinical joint pain research. *J. Neurosci. Methods* **300**, 92–102 (2018).

138. Nordquist, R. E., Meijer, E., van der Staay, F. J. & Arndt, S. S. Pigs as model species to investigate effects of early life events on behavioral and neurological function. in *Animal models for the study of human disease* 1003–1030 (2017).

139. Zebunke, M., Kreiser, M., Melzer, N., Langbein, J. & Puppe, B. Better, Not Just More—Contrast in Qualitative Aspects of Reward Facilitates Impulse Control in Pigs. *Front. Psychol.* **9**, (2018).

140. Rice, F. L. *et al.* Human-like cutaneous neuropathologies associated with a porcine model of peripheral neuritis: A translational platform for neuropathic pain. *Neurobiol. Pain* **5**, 100021 (2019).

141. Kennedy, W. R. *et al.* A Randomized, Controlled, Open-Label Study of the Long-Term Effects of NGX-4010, a High-Concentration Capsaicin Patch, on Epidermal Nerve Fiber Density and Sensory Function in Healthy Volunteers. *J. Pain* **11**, 579–587 (2010).

142. Landmann, G. *et al.* Short lasting transient effects of a capsaicin 8% patch on nociceptor activation in humans. *Eur. J. Pain* **20**, 1443–1453 (2016).

143. Lo Vecchio, S., Andersen, H. H. & Arendt-Nielsen, L. The time course of brief and prolonged topical 8% capsaicin-induced desensitization in healthy volunteers evaluated by quantitative sensory testing and vasomotor imaging. *Exp. Brain Res.* **236**, 2231–2244 (2018).

144. Nielsen, T. A., Eriksen, M. A., Gazerani, P. & Andersen, H. H. Psychophysical and vasomotor evidence for interdependency of TRPA1 and TRPV1-evoked nociceptive responses in human skin: an experimental study. *PAIN* **159**, 1989–2001 (2018).

145. Koppert, W., Brueckl, V., Weidner, C. & Schmelz, M. Mechanically induced axon reflex and hyperalgesia in human UV-B burn are reduced by systemic lidocaine. *Eur. J. Pain* **8**, 237–244 (2004).

146. Sycha, T. *et al.* Rofecoxib attenuates both primary and secondary inflammatory hyperalgesia: a randomized, double blinded, placebo controlled crossover trial in the UV-B pain model: *Pain* **113**, 316–322 (2005).

147. Weinkauf, B., Main, M., Schmelz, M. & Rukwied, R. Modality-Specific Nociceptor Sensitization Following UV-B Irradiation of Human Skin. *J. Pain* **14**, 739–746 (2013).

148. Lötsch, J. *et al.* Quantitative sensory testing response patterns to capsaicin- and ultraviolet-B–induced local skin hypersensitization in healthy subjects: a machine-learned analysis. *PAIN* **159**, 11–24 (2018).
149. Rössler, B. *et al.* Central origin of pinprick hyperalgesia adjacent to an UV-B induced inflammatory skin pain model in healthy volunteers. *Scand. J. Pain* **4**, 40–45 (2013).
150. Andresen, T. *et al.* Intradermal Injection with Nerve Growth Factor: A Reproducible Model to Induce Experimental Allodynia and Hyperalgesia. *Pain Pract.* **16**, 12–23 (2016).
151. Papagianni, A., Siedler, G., Sommer, C. & Üçeyler, N. Capsaicin 8% patch reversibly reduces A-delta fiber evoked potential amplitudes: *PAIN Rep.* **3**, e644 (2018).
152. Doll, R. J. *et al.* Responsiveness of electrical nociceptive detection thresholds to capsaicin (8 %)-induced changes in nociceptive processing. *Exp. Brain Res.* **234**, 2505–2514 (2016).
153. Maihöfner, C., Ringler, R., Herrndobler, F. & Koppert, W. Brain imaging of analgesic and antihyperalgesic effects of cyclooxygenase inhibition in an experimental human pain model: a functional MRI study: Functional imaging of COX inhibition. *Eur. J. Neurosci.* **26**, 1344–1356 (2007).

Box.1| Keywords and criteria used for literature search

Combination of keywords I:

- "pain model" (everywhere) **AND**
- pig **OR** porcine **OR** swine **OR** piglet (in title/abstract/keywords) **AND**
- **NOT** "guinea pig" (in title/abstract/keywords)

Combination of keywords II:

- hyperalgesia (everywhere) **AND**
- "pig model" **OR** "porcine model" **OR** "swine model" (in title/abstract/keywords) **AND**
- **NOT** "guinea pig" (in title/abstract/keywords)

No limitations were set with regard to language, publication type, year and status. The last literature search was performed in March 2021. Titles and abstracts were retrieved for all the identified literature and were evaluated using the following exclusion criteria:

- Other animal species
- Human studies
- In vitro studies
- Not peer-reviewed material
- Not using a pain model (for example a disease model where pain was not evaluated, or pain assessment in otherwise healthy pigs)

Full-text articles were retrieved for all included literature. The articles were reviewed once more using the same exclusion criteria. Relevant references within the included literature were also retrieved and assessed using the same exclusion criteria.

Fig. 1| Flowchart of the studies identified, assessed, included and excluded with reasons for exclusion.

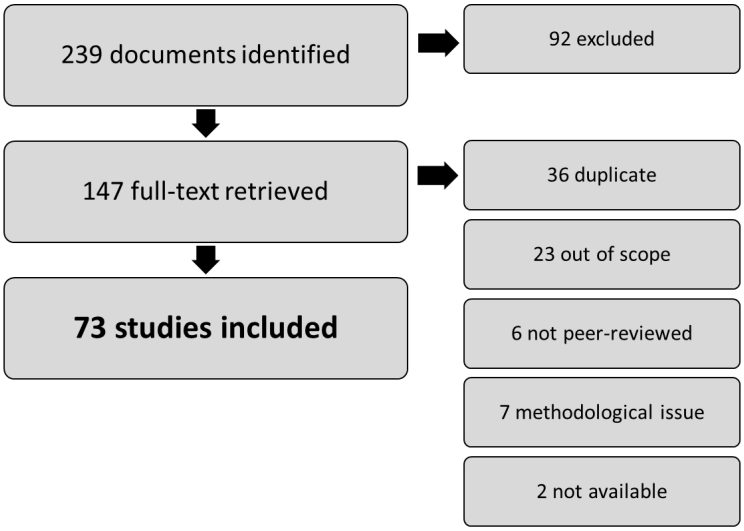


Fig. 2| There is an increasing interest in research to investigate pain in pigs. The studies identified in the present search were categorized in four groups and results are presented by number of publications per 5 years.

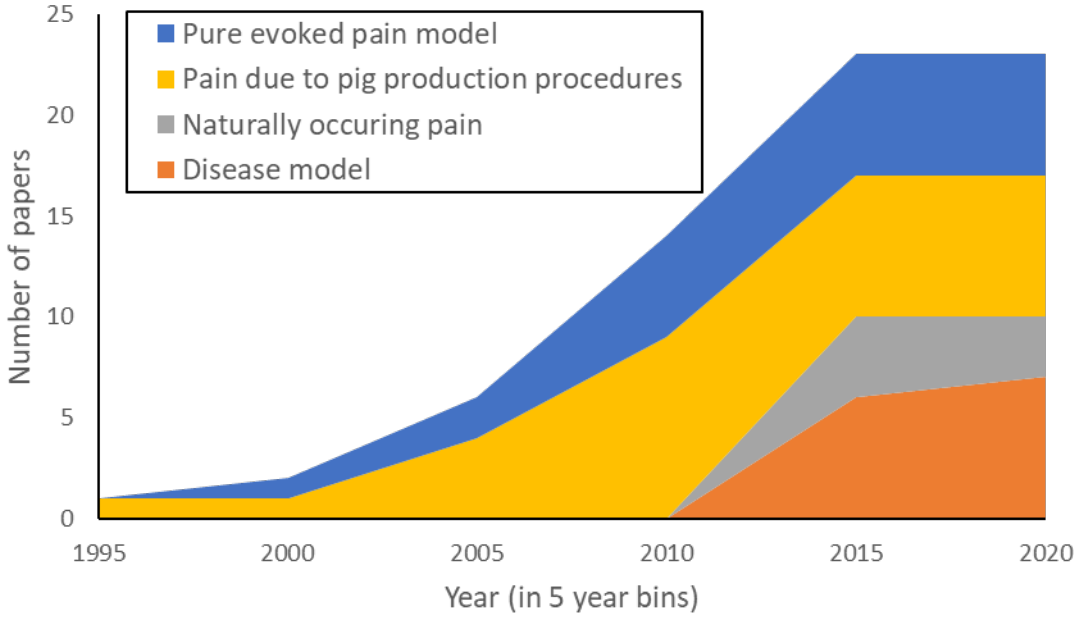


Fig. 3| The duration and intensity of mechanical sensitization after surgery[51–53], after application of an inflammatory agent[54,58–60,62] or in irreversible models of pain[34,64,98]. The intensities were calculated using mechanical sensitization levels; 0 represents no mechanical sensitization and 1 represents a maximal increase in mechanical pressure sensitivity**. a,** Mechanical sensitization was comparable for most inflammatory models but insignificant for the capsaicin patch**. b,** Mechanical sensitization when pressure was applied to the wound was severe and prolonged in surgical models, and the values had not returned to normal at the end of the studies.**c,** Mechanical sensitization was severe relatively quickly after surgery for PNT compared with the nerve crush models. Mechanical sensitization was also observed up to 4 months after tail resection; measurements were conducted 1, 8 and 16 weeks after resection.*In the NGF model withdrawal thresholds were not used. Considering withdrawal a reflex, peripheral sensitization is the main factor contributing to its change; therefore, intensity was calculated as the estimated change in withdrawal thresholds based on the change in the area of erythema in the NGF model relative to the UV-B models. (a.u.) stands for arbitrary units, as the data is normalized to allow comparison.

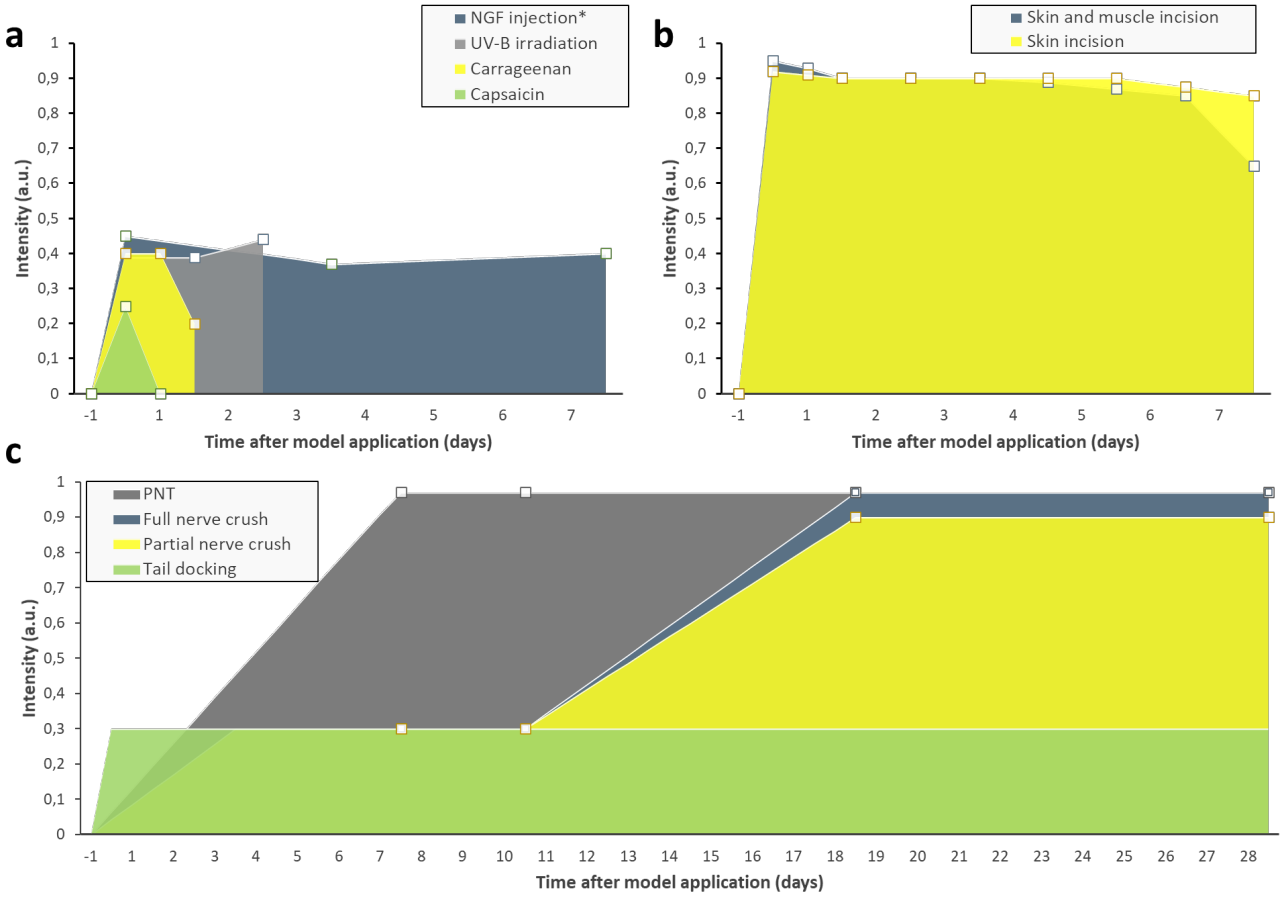


Fig. 4| The duration and intensity of mechanical sensitization for lameness induced by kaolin [71], amphotericin B [67,69,70] and natural lameness [105]. For lameness induced by kaolin and natural lameness, measurements were conducted 3 days in a row. In the lameness models, mechanical sensitization after induction was compared with baseline, while for natural lameness it was compared with a control group.

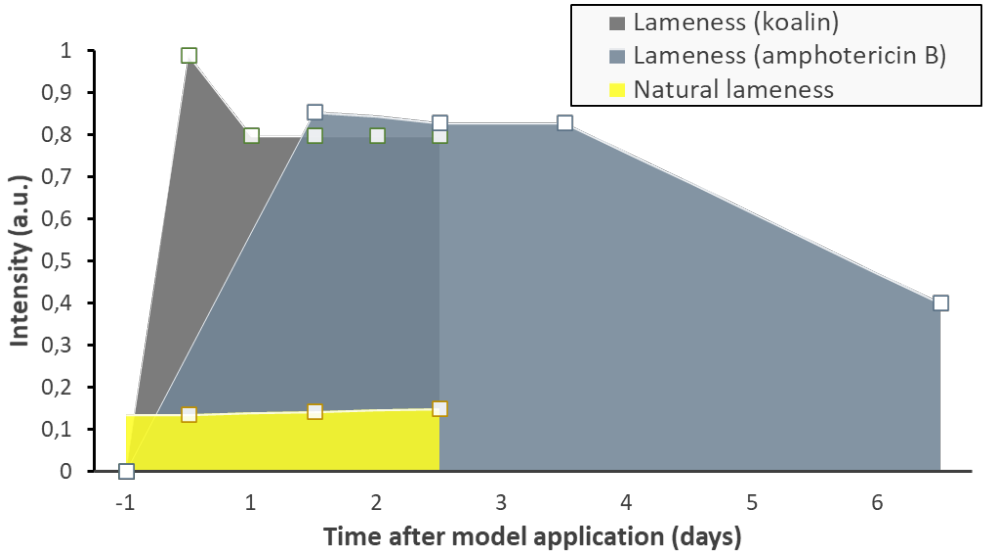
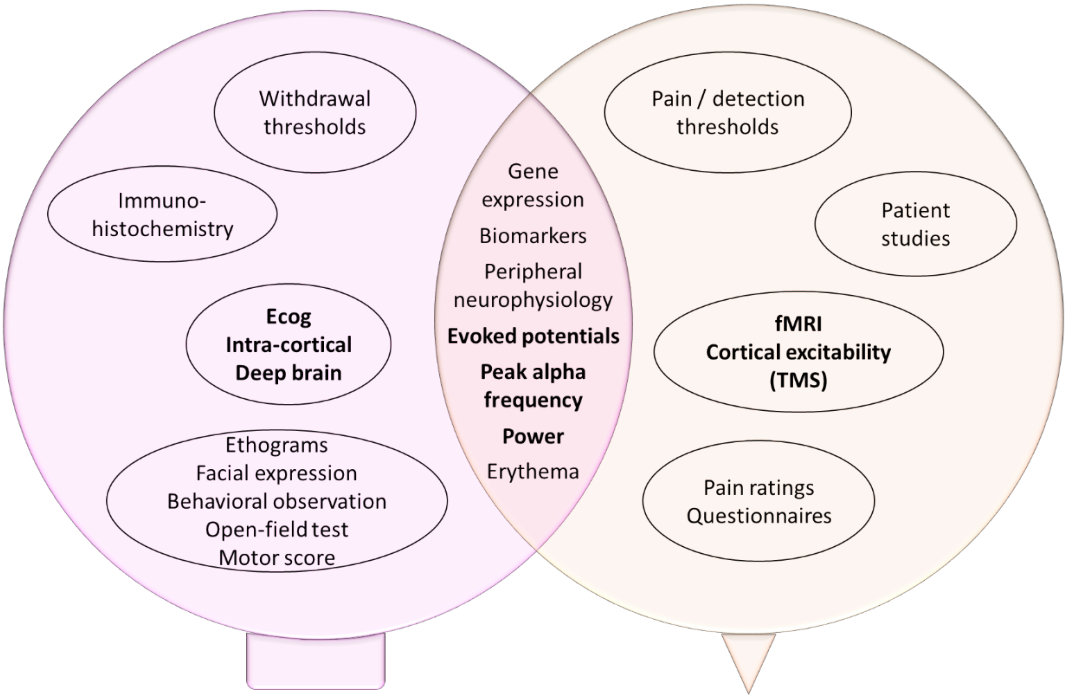


Fig. 5|Overview of the different identified pain assessment methods. Some pain assessment methods can be used either in pigs or in humans while others can be used in both species. Future translational pain research may be optimized by directly comparing information obtained from assessment techniques that can be applied in both species, as well as by seeking unique and complementary knowledge with techniques that can only be used in one species. For example, in humans it is possible to get detailed information about the pain experience, while in pigs more invasive measurements (e.g. intra-cortical) and models (e.g. nerve damage model) can be applied. ECoG; electrocorticography



1

Table 1| Categorization of the included literature

Pain model type	Number of articles	Number of models
I. Surrogate pain models	20	10
II. Disease models	13	4 (lameness was also a naturally occurring pain model)
III. Pain due to pig production procedures	30 (1 shared with natural pain)	6
IV. Naturally occurring pain	8 (1 shared with production procedures)	3 (lameness was also a disease model)

Adapted from [42]. Three papers were reviews on pain assessment [27,42,43]; the relevant original manuscripts presented in these reviews were identified by searching the reference lists. Multiple papers may have used the same pig model, 23 models were identified from the included literature.

Table 2| The pain duration and manifestations of each identified pain model.

	Pain model	Duration	Manifestations	Ref.
Surrogate pain models	Full surgery	At least 2 days	Lameness, vocalization, physiological and other behavioral indicators	47,48
	Skin incision	At least 1 week	Mechanical sensitization, increased (social) behavior score and increased vocalization up to 3 hours after surgery	51–53
	Skin and muscle incision and retraction	At least 1 week	Mechanical sensitization, increased (social) behavior score and increased vocalization up to 3 hours after surgery	51,52
	Capsaicin	30 minutes	Thermal sensitization only in small pigs	62
	Carrageenan	1- 3 days	Mechanical sensitization (up to 24 hours) and lesions (3 days)	60
	UV-B	At least 2 days	Mechanical and thermal sensitization (withdrawal and erythema)	58,59
	NGF	At least 1 week	Acute peripheral heat and prolonged chemical sensitization, reduction in activity dependent slowing	54,57
		At least 3 weeks	Decrease in mechanical activation threshold, facilitation of post-spike excitability and increase in receptive field	56,109
	Partial nerve crush	At least 4 weeks	Mechanical sensitization from day 18, allodynia from 1 week, behavioral indicators, motor deficit up to 10 days	140
	Full nerve crush	At least 3 weeks	Mechanical sensitization from day 18, allodynia from 1 week, behavioral indicators, motor deficit up to 10 days	140
	CFA soaked sutures (PTN model)	At least 4 weeks	Mechanical sensitization, allodynia, behavioral indicators, different open-field pattern	34,140
	Surgical tail amputation	1-16 weeks	Mechanical sensitization, gene expression	90,99
Disease models	Femoral fracture	NA (No sham or baseline)		75
	Lameness - Koalin	At least 2 days	Mechanical sensitization	71
	Lameness - Amphotericin B	6 days	Mechanical and thermal sensitization (3 days), behavioral indicators (3 days)	68–70
	Osteoarthritis	At least 35 weeks (progressive)	Lesions, gait alterations.	72,73
	Neurofribromatosis	At least 24 months (genetic modification)	Mechanical and thermal sensitization	125
Pig production procedures	Tail docking	During and acutely after	Increased cortisol, vocalization and other behavioral indicators	44,95, 97
	Teeth clipping	Acute (up to 120s)	Teeth champing	44
	Ear notching & tagging	Up to 3 hours	Behavioral indicators, in particular head shaking and ear scratching	44,100
	IP transponders	Up to 3 hours	Behavioral indicators	100
	Castration	During	Vocalization, defense movement	76–81,83
		Acute (up to 60 minutes)	Increased serum cortisol, longer navigation time in handling chute	80,83, 86,87, 134
		Intermediate (3-4 hours)	Increased eye and rectal temperature, behavioral indicators: prostration, trembling, stiffness, scratching	78,80, 86,87, 89
		Prolonged (up to 4 days)	Behavioral indicators: scratching and tail wagging	85,89
	Castration	Conflicting	Behavioral indicators: Lying, sitting, standing, walking, huddling up, time/activity at the udder, isolating from pen mates and/or sow	78,80, 85,86, 89
Natural models	Farrowing	24 hours	Behavioral indicators	102
	Lameness	Unknown	Mechanical hyperalgesia compared to non-lame limbs	105
	Ulcers	Unknown	Behavioral indicators	107

Models are subdivided into 4 categories (surrogate pain, pig production, natural pain and disease models) and ordered according to their reported duration. NA; not available

Table 3| Overview of assessment methods used in human pain models (only pain induced by inflammatory agents) and animal pain models (only surrogate pain models).

	ASSESSMENT TYPE	ANIMAL ASSESSMENT	HUMAN ASSESSMENT	SPECIES	REFERENCES
EVOKED RESPONSES	THERMAL HYPERALGESIA	Heat withdrawal latency	Heat pain threshold	Both	62,113,141–150
			Cold pain threshold	Human	113,142,143,148,149
			Warm and cold detection	Human	113,142,143,148,149,151
		NA	Heat pain rating	Human	143,144,147
			Alternating hot and cold	Human	113,148,151
		Thermally induced flare area*	NA	Pig	54,58
		Thermal C-fiber activation threshold**	Thermal C-fiber activation**	Both	56,109 &
	MECHANICAL HYPERALGESIA	Mechanical withdrawal threshold	Pressure pain threshold, pinprick	Both	60,62,98,109,111–113,115,116,118,119,121,141,143,146–151
		NA	Mechanical detection, vibration detection	Human	113,141,143,148,151
			Temporal summation	Human	113,119,148,150
		Mechanically induced flare area*	NA	Pig	54,58
		Mechanical C-fiber activation threshold**	Mechanical C-fiber activation**	Both	56,109
	CHEMICAL	Chemically induced flare area*	NA	Pig	54
	ALLODYNIA	NA	Allodynic area (pin prick)	Human	113
		Allodynia (feather)	Allodynia (brush)	Both	34,64,113,121,131,149,150
	ELECTRICALLY EVOKED RESPONSES	Electrical C-fiber activation threshold**	Electrical activation and pain threshold	Both	56,57,109,110,142,147
		Electrically induced flare area*	Electrically induced flare area*	Both	54,110,147
			Electrical perception threshold	Human	110,147,152
		NA	Electrical pain rating		110,142,144
NON- EVOKED MEASURES	EXPERIENTIAL MEASURES	Ethogram - body move, rubbing, muscle twitch	Pain ratings	Both	59,62,111,114,116,118,120,121
		Observation of pain-related behavior	McGill pain questionnaire, pain drawing, headache diary and other questionnaires	Both	34,46–48,51,63,64,111–117
		Open-field test	NA	Pig	63
		Food consumption	NA	Pig	46
	MOTOR FUNCTION	Score, activity percentage, time between movements	Likert scale	Both	34,46,53,64,111,112,114–116,118,119
PHYSIOLOGICAL MEASURES	PERIPHERAL NEUROPHYSIOLOGY	Activity-dependent slowing, conduction velocity, recovery rate	Activity-dependent slowing, conduction velocity, recovery rate	Both	56,57,109
	CENTRAL NEUROPHYSIOLOGY	NA	EEG / evoked potentials	Human	112,114,115,142,151
		NA	EEG / peak alpha frequency	Human	120
		NA	fMRI	Human	153
		NA	Cortical excitability	Human	112,114,115
	BIOMARKERS	Immunohistochemistry	NA	Pig	34,48–50
		Blood sampling	NA	Pig	46,48
		Gene expression	NA	Pig	61,99
		Skin biopsy	Skin biopsy	Both	56,64
	PHYSIOLOGICAL PARAMETERS	Heart rate, blood pressure, respiratory rate, core temperature	NA	Pig	47

*Induction of erythema or flare occurs via the axon reflex; it is therefore a measure of nociceptive activation but not of pain.

**C-fiber activation threshold is also a measure of nociceptive activation; it is not clear how much activation is required for detection and pain thresholds.

1 [&]note: thermal activation was used in [109], but as a part of the "marking" technique

2 NA; not available

3

Supplementary Table 1 **Bias assessment for studies included in the quantitative comparison.**

Ref.	Quality score	Number of animals per group	Control group without pain model	Blinding	Randomization	Model	Follow up period
[1]	3	min 13	Yes	No	Yes	Tail docking	8-16w
[2]	2	8 (1 group of 4)	No*	Yes	Yes	Incision (SMIR)	7d
[3]	0	6	No*	No	No	Incision (SI and SMIR)	24h
[4]	2	5	Internal control (same animal)*	Yes	No	Incision (SI and SMIR)	7d
[5]	4	6	Yes	Yes	Yes	Nerve damage (PNT)	28d
[6]	3	min 3	Yes	Yes	No	Nerve damage (PNT, partial and full nerve crush)	28d
[7]	2	6	Yes	No	No	Nerve damage (PNT)	21+7d
[8]	1	min 7	Internal control (same animal)	No	No	UVB	48h
[9]	3	16	Yes	No	Yes	UVB	48h
[10]	2	8	Internal control (same animal)	No	Yes	NGF	7d
[11]	1	8	Cross-over + internal	No	No	Carrageenan	4h+24h
[12]	2	24	Internal control (same animal)	No	Yes	Capsaicin	0.5h
[13]	2	12	No*	Yes	Yes	Koalin	44h
[14]	3	4	Internal control (same animal)*	Yes	Yes	Amphotericin B	6d
[15]	3	24	Internal control (same animal)	Yes	Yes	Amphotericin B	6d
[16]	2	4	Internal control (same animal)*	No	Yes	Amphotericin B	6d
[17]	2	14	Yes	No	No	Lameness (natural)	3d

*These studies investigated a pharmacological compound, the control group received the pain model without pharmacological treatment.

SMIR; Skin and muscle incision and retraction

SI; Skin incision

PNT; Peripheral neuritis trauma

References

1. Di Giminiani, P. *et al.* Characterization of short- and long-term mechanical sensitisation following surgical tail amputation in pigs. *Sci. Rep.* **7**, 4827 (2017).
2. Wilsey, J. T. & Block, J. Sustained analgesic effect of clonidine co-polymer depot in a porcine incisional pain model. *J. Pain Res.* **Volume 11**, 693–701 (2018).
3. Castel, D., Sabbag, I. & Meilin, S. The effect of local/topical analgesics on incisional pain in a pig model. *J. Pain Res.* **Volume 10**, 2169–2175 (2017).

4. Castel, D., Willentz, E., Doron, O., Brenner, O. & Meilin, S. Characterization of a porcine model of post-operative pain: A pig model of incisional pain. *Eur. J. Pain* **18**, 496–505 (2014).
5. Castel, D., Sabbag, I., Brenner, O. & Meilin, S. Peripheral Neuritis Trauma in Pigs: A Neuropathic Pain Model. *J. Pain* **17**, 36–49 (2016).
6. Rice, F. L. *et al.* Human-like cutaneous neuropathologies associated with a porcine model of peripheral neuritis: A translational platform for neuropathic pain. *Neurobiol. Pain* **5**, 100021 (2019).
7. Castel, D., Sabbag, I., Nasaev, E., Peng, S. & Meilin, S. Open field and a behavior score in PNT model for neuropathic pain in pigs. *J. Pain Res.* **Volume 11**, 2279–2293 (2018).
8. Rukwied, R., Dusch, M., Schley, M., Forsch, E. & Schmelz, M. Nociceptor sensitization to mechanical and thermal stimuli in pig skin in vivo. *Eur. J. Pain* **12**, 242–250 (2008).
9. Di Giminiani, P., Petersen, L. J. & Herskin, M. S. Characterization of nociceptive behavioural responses in the awake pig following UV-B-induced inflammation: UV-B induced hyperalgesia in the pig. *Eur. J. Pain* **18**, 20–28 (2014).
10. Rukwied, R. *et al.* Nerve growth factor-evoked nociceptor sensitization in pig skin in vivo. *J. Neurosci. Res.* NA-NA (2010) doi:10.1002/jnr.22351.
11. Sandercock, D. A. *et al.* Development of a mechanical stimulator and force measurement system for the assessment of nociceptive thresholds in pigs. *J. Neurosci. Methods* **182**, 64–70 (2009).
12. Di Giminiani, P., Petersen, L. J. & Herskin, M. S. Capsaicin-induced neurogenic inflammation in pig skin: A behavioural study. *Res. Vet. Sci.* **96**, 447–453 (2014).
13. Fosse, T. K. *et al.* Ketoprofen in piglets: enantioselective pharmacokinetics, pharmacodynamics and PK/PD modelling: Pharmacokinetics and pharmacodynamics of ketoprofen in piglets. *J. Vet. Pharmacol. Ther.* **34**, 338–349 (2011).
14. Tapper, K. R. *et al.* Pressure algometry and thermal sensitivity for assessing pain sensitivity and effects of flunixin meglumine and sodium salicylate in a transient lameness model in sows. *Livest. Sci.* **157**, 245–253 (2013).
15. Mohling, C. M. *et al.* Evaluation of mechanical and thermal nociception as objective tools to measure painful and nonpainful lameness phases in multiparous sows. *J. Anim. Sci.* **92**, 3073–3081 (2014).
16. Pairis-Garcia, M. *et al.* Measuring the efficacy of flunixin meglumine and meloxicam for lame sows using nociceptive threshold tests. *Anim. Welf.* **23**, 219–229 (2014).
17. Nalon, E. *et al.* Mechanical nociception thresholds in lame sows: Evidence of hyperalgesia as measured by two different methods. *Vet. J.* **198**, 386–390 (2013).