Pressure mat analysis for detecting and quantifying lameness in pigs

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Pressure mat analysis for detecting and quantifying lameness in pigs

Drukmatanalyse voor het opsporen en kwantificeren van kreupelheid bij varkens

(met een samenvatting in het Nederlands)

Proefschrift

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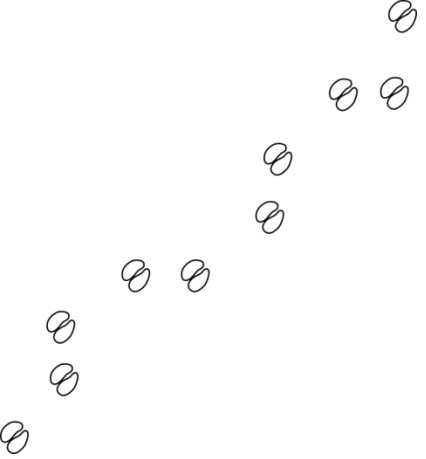

1

General Introduction

Locomotion (from the Latin: *locos* "from a place" and *motionem* "motion, a moving"), or the ability to move from one place to another, is an essential ability for the survival of many animals. Locomotion may for example be needed to evade predators, to find or catch food and to find a mate. Although there are some species that transport themselves passively (the environment provides transport, for example: ballooning or kiting in spiders) most of our domesticated mammals propel themselves actively by the use of four limbs.

1.1: Normal gait

Locomotion in most mammals is achieved through gait. Gait is defined as "a complex and strictly coordinated, rhythmic and automatic movement of the limbs and the entire body of the animal, which results in the production of progressive movements" (Back and Clayton, 2013a). These automated movements of the limbs are repeated in a cyclic manner, and one such cycle of movements, and the footfalls associated with these movements, is called a stride. Depending of the number of footfalls that can be heard during one stride, a gait is called two-, three-, or four-beat. Additionally, in quadruped gait, footfalls of the front (left and right) and hind (left and right) limb pair can either be evenly spaced in time or unevenly spaced in time. These are called symmetrical or asymmetrical gaits respectively (Robilliard et al., 2007).

Quadruped gait is studied most extensively in the horse, in which many gaits are distinguished. Two of these equine gaits are comparable to gaits in pigs, namely the walk and the trot. Furthermore, pigs can use a bounding gait which resembles the gallop in horses. The walk and trot are classified as symmetrical gaits, while the "gallop" is usually asymmetrical. At the walk, four beats are discernable and a suspension phase, in which all four legs are off the floor, is not present. In contrast, the trot is a two-beat gait with a suspension phase (Back and Clayton, 2013a). The bounding "gallop" of the pig is somewhat variable in footfall patterns, and although a clear suspension phase is difficult to see, gait visualization using a pressure mat shows that this suspension phase is present (personal observation).

1.2: Abnormal gait: Lameness

Locomotion is achieved through an integrated effort of nervous tissue, muscles, bones, joints, tendons and specialized skin. When there is a lesion in one or more of these tissues, lameness may occur. The lesion may induce pain, or may cause mechanical obstruction, neurologic dysfunction or vascular obstruction. All of these may cause altered locomotion.

1.2.1 Definition of lameness

Lameness is defined as an incapability of normal locomotion; a deviation of the normal gait (Studdert et al., 2012). In addition, some definitions also include a normal degree of alertness and normal coordination in the other unaffected limbs (Straw et al., 1999), limping or decreased weight bearing (Radostits et al., 2007).

Central to all definitions of lameness is a deviation of normal gait. In the majority of cases, this deviation of normal gait arises as the animal attempts to avoid pain, for example by redistributing its weight away from the lame limb. There are, however, also other causes than pain for an altered gait. Mechanical impairments may for example arise when aberrant bone formation may restrict normal range of motion. Mechanical impairments are not by definition painful, although they often occur in conjunction with painful conditions. A defect in the nervous system that steers locomotion also does not necessarily mean that the animal experiences pain, but it may cause altered gait. In conclusion, in the majority of cases lameness is caused by painful conditions; there are however exceptions, which means that lameness cannot be directly translated to pain.

1.2.2 Consequences of lameness

Lameness may have negative consequences on both **animal welfare** and **economic profit**.

Welfare in animals is often defined in terms of the "five freedoms of Brambell" (Brambell Comittee, 1965). These five freedoms outline requirements that are considered necessary for adequate animal welfare, and are often used as a framework for discussions on animal welfare. The five freedoms stated in the original Brambell report are: "(1) Freedom from hunger, thirst, (2) freedom from discomfort, (3) freedom from pain, injury or disease, (4) freedom to exhibit normal behaviour and (5) freedom from fear and distress. Recently, more attention is paid to positive indicators of welfare, and the description of welfare in terms of adaptability. This means that an animal is in a positive state of welfare when it is able to "*adequately react to hunger, thirst, thermal or physical discomfort, injuries or diseases, fear and chronic stress, and thus has the freedom to display normal behavioural patterns that allow the animal to adapt to the demands of the prevailing environmental circumstances and enable it to reach a state that it perceives as positive*" (Ohl and Van Der Staay, 2012). Welfare in lame animals is generally considered to be impaired (Whaytt et al., 2003) for several reasons (Anil et al., 2009).

First of all, lameness is often associated with painful conditions. Pain is difficult to study in non-verbal subjects such as pigs. However, most orthopedic conditions that cause lameness in animals, for example osteoarthritis (OA) and osteochondrosis (OC), also

occur in humans. Human patients, in contrast to animals, can self-report pain and generally rate these conditions as painful. If we apply the analogy principle, we may therefore conclude that these conditions also cause pain in animals. Often, the range of joint motion is also affected in these conditions (Dieppe and Lohmander, 2005; McCoy et al., 2013), which further adds to the characteristic phenotypical manifestation of lameness. The consequences of orthopedic pain in human patients are that normal physical activities often become difficult to perform and the quality of life, as perceived by the patient, decreases (Cook et al., 2006; Rakel et al., 2015). In humans, chronic pain states also often commonly occur together with affective disorders such as depression (Bair et al., 2003; Li, 2015; Stubbs et al., 2016), further decreasing quality of life.

In animals, measuring, and often even recognizing, pain is challenging (Anil et al., 2002; Barnett, 1997; Cobianchi et al., 2014; Prunier et al., 2013; Rutherford, 2002; Walker et al., 2011; Weary et al., 2006). Even so, based on analogies in anatomical, (patho-) physiological, biochemical, and behavioral responses to noxious stimuli, most students of animal health, behaviour and welfare consider at least vertebrate animals capable of experiencing pain in a way similar to humans. Lameness in animals is often considered to be guarding behaviour, and therefore a direct consequence of pain (Gregory et al., 2013).

Apart from pain, an animal that is lame may also encounter other welfare issues. Hunger or thirst may be the result of a decreased ability to compete for, reach or consume food and water (Galindo and Broom, 2002; Weeks et al., 2000) and thus the ability of an animal to adequately react to these negative stimuli is impaired, resulting in decreased welfare. This effect is also observed in lame pigs, which may have difficulties going to the feeding and drinking stations, resulting in hunger and thirst (Anil et al., 2009; Madec et al., 1986). Additionally, group-housed pigs may experience difficulties in maintaining social rank within a group (Cornou et al., 2008).

Economic profit for the farmer may be decreased due to increased costs for medication (Christensen et al., 1994), lower feed intake and therefor slower growth in affected animals (Munsterhjelm et al., 2015) and increased mortality or culling of lame pigs (Engblom et al., 2008; Jensen et al., 2010).

1.2.3 Prevalence

Prevalence studies on lameness in weaned pigs are scarce. Most studies on pig lameness focus on sows. Although lameness in weaned piglets and finishing pigs has not been studied as extensively as lameness in sows, it is a very common condition in these animals as well. In a cross-sectional study in the U.K., the prevalence of clinical lameness in finishing pigs was 19.7% (KilBride et al., 2009). Other studies found lower prevalences, some as low as 2% (Petersen et al., 2008; van den Berg et al., 2007). Unfortunately,

studies are difficult to compare since sampling methods, scoring systems and cutoff points to detect lameness vary widely.

1.2.4 Etiology

Lameness may be caused by several conditions: infectious agents, physical injury and several manifestations of osteochondrosis (Jensen and Toft, 2009; Wells, 1984) and may be located in nervous tissue, muscles, bones, joints and/or tendons.

Inflammation of one or more joints is commonly found in weaned piglets and finishing pigs and may be caused by several infectious agents, such as Streptococcus spp., Mycoplasma spp., Haemophilis parasuis and Erysipelothrix rhusiopatiae (Jensen and Toft, 2009; Nielsen et al., 2001; Wells, 1984). Infections in other structures, such as connective tissue, bones, claws, tendons, nervous tissue and muscles may also cause lameness but are less common. Injuries to the claws are very common (93.8 % in one study) and are often the result of slippery or otherwise unsuitable flooring (Mouttotou et al., 1997). Osteochondrosis is a disturbance of enchondral ossification in growing pigs and, if severe enough, can cause lameness. It is a multifactorial disease and may be influenced by genetics, dietary factors, growth rate, anatomic features and trauma (De Koning et al., 2012; Ytrehus et al., 2007)

1.2.5 Risk factors

Several risk factors have been implicated in the development of lameness. Housing, and especially flooring, is recognized as an important risk factor for lameness in finishing pigs (Guy et al., 2002; KilBride et al., 2009; Scott et al., 2006). Different housing systems may predispose for osteochondrosis (Jorgensen, 2002; Van Grevenhof et al., 2011), injuries and claw lesions. (Cagienard et al., 2005; Guy et al., 2002; Jorgensen, 2002; Lyons et al., 1995; Van Grevenhof et al., 2011) Finishing pigs grow extremely fast and therefore correct feed composition is critical. The amounts of minerals such as calcium and phospohorus need to be balanced in order for pigs to grow healthy bones. If bone growth cannot occur in optimal conditions, defects and lameness may occur. Hereditary factors have been implicated in the development of osteochondrosis and in so-called “leg weakness”, a clinical syndrome that involves both poor conformation and locomotion (Jorgensen and Andersen, 2000; Ytrehus et al., 2004).

1.2.6 Treatment options

Two groups of pharmaceutical treatment options are registered for use in lame pigs in the EU: antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs).

Antibiotics are widely used in lame pigs. It is common to have antibiotics in on-farm treatment protocols for lame pigs. Antibiotics can be very effective against several infectious causes of arthritis, especially when they are used early in the disease process (Radostits et al., 2007). Unfortunately, often no investigation into the causative agent for lameness in pigs is performed, which means antibiotic treatment may be unnecessary or ineffective. An example is the common use of penicillin in Mycoplasma Hyosynoviae arthritis (Nielsen et al., 2001).

NSAIDs have both anti-inflammatory and analgesic properties. There is a large body of evidence suggesting their effectiveness in the treatment of musculoskeletal disease in humans and other animals (Deeks et al., 2002; Flower et al., 2008; Gunew et al., 2008; Payne-Johnson et al., 2015; Walliser et al., 2015), and some evidence that they are effective for the treatment of lame pigs (Friton et al., 2002; Mustonen et al., 2011). Although NSAIDs are registered for use in lame pigs, their use is still not common practice (Flecknell, 2008). There may be several reasons for this underuse, including economic considerations, difficulty in identifying animals in need of treatment, culture or tradition and uncertainty about withdrawal periods (Hewson et al., 2007; Ison and Rutherford, 2014).

Apart from pharmacological interventions, in sows preventive or curative claw trimming is possible (Jorgensen, 1999), it is however rarely practiced. Animals that are unable to maintain themselves within the group are often moved to a sick bay.

1.3: Quantifying lameness

Due to the high prevalence of lameness in pigs and the consequences for both pigs and farmers, it is important to develop new or better interventions. When assessing the effect of an intervention, it is important to be able to a) detect the problem and b) quantify it.

Traditionally, lameness assessment is carried out by a veterinarian or farmer/owner. Many parameters such as weight bearing, posture, step length and timing of footfalls are assessed at once, some consciously and some subconsciously. Although this method is fast and cheap, it suffers from observer bias (Petersen et al., 2004)

To quantify lameness more objectively, and to be able to separate different aspects of lameness more clearly, several methods have been developed. They can be divided in **kinetics**, methods that assess the forces that affect motion and **kinematics**, methods that assess the temporospatial and geometric characteristics of motion (McLaughlin, 2001). The most well-known kinetic method is force plate analysis. Kinematic analysis in animals is most often performed using markers and high-speed cameras. A relatively new method that can capture several kinetic and kinematic parameters is pressure mat analysis. The advantages and drawbacks of these systems will be discussed below.

Systems may further be divided into wearable systems and non-wearable systems (Muro-de-la-Herran et al., 2014). An example of a wearable system that has been used in gait analysis of horses is accelerometry/inertial sensors (Keegan et al., 2012). Non-wearable systems include floor-mounted systems such as force plates and pressure mats, and methods that rely on image processing to obtain information on gait.

1.3.1 Visual scoring

The simplest, fastest and least expensive method to quantify lameness is visual scoring. Scores can be assigned on either numerical scales or visual analogue scales. Numerical rating scales (NRS) sort degrees of lameness into discrete, ordered categories using descriptions of the gait of an animal (D'Eath, 2012; Dewey et al., 1993; Grégoire et al., 2013; Karlen et al., 2007; Karriker et al., 2013). Some NRS do not only include gait characteristics, but also look at anatomical features or behaviour that may be associated with lameness (De Koning et al., 2012; Main et al., 2000). The number of categories in a NRS varies, but it has been suggested that scales with less than five categories may not be discriminative enough when used for lameness scoring in sows (Nalon et al., 2014). Visual analogue scales (VAS) are typically a 100 mm-long horizontal line with descriptors at either end. Raters indicate the lameness score by placing a mark along the line. A study in lame dogs showed that VAS correlated better with objective force plate analysis of lame dogs than NRS did (Quinn et al., 2007). Inter- and intraobserver repeatability of VAS scores of lame sows was higher for VAS than for a 2-point NRS and comparable to a 5-point scale (Nalon et al., 2014).

Visual lameness scoring in pigs can be challenging for several reasons. First of all, pigs are prey animals and tend to hide signs of lameness (Weary et al., 2008). Also, pigs are herd animals and it is therefore in many countries legally required to keep them in groups. This makes scoring of the animal in its home pen difficult. An obvious solution to this problem would be to isolate the animal in order to observe its gait. Unfortunately, animals that are isolated from their group are usually anxious and tend to hide lameness. Apart from their behavioural characteristics and resulting housing requirements, pigs are also difficult to score visually due to their anatomical properties. Dogs, cattle and especially horses have a long neck which means that head bobbing associated with lameness is easily observed. Pigs have a short neck that makes any head bobbing that may be present much less obvious (Main et al., 2000a).

Visual scoring has some important observer-based drawbacks. It is subjective (Arkell et al., 2006) and only has moderate inter-observer agreement (D'Eath, 2012; Keegan, 2007; Main et al., 2000; Petersen et al., 2004). Furthermore, visual scores do not always correlate well with either objective force plate measurements or joint pathology (Etterlin et al., 2015; Quinn et al., 2007). Trained observers generally obtain more repeatable results

than untrained observers (Main et al., 2000), and repeatability increases with increasing lameness severity.

1.3.2 Force plate analysis

Force plates have been used to obtain kinetic information on gait since the late 1960's. They are still considered the "gold standard" when it comes to kinetic gait analysis. Force plates consist of a platform that is mounted onto at least four piezo-electric transducers or strain gauges. When an animal steps on the plate, the magnitude and direction of the force that is exerted onto the platform is measured and converted into an electrical signal.

Force plates require a solid concrete foundation and their use is therefore mainly restricted to gait laboratories. The force plate is not able to distinguish between feet that are placed simultaneously on the plate. This means that measurements in which two or more feet are in contact with the plate simultaneously have to be discarded and numerous trails need to be collected, thus making data acquisition time-consuming. It also means that, when comparing feet or animals to each other, either all the factors that introduce variability between trials need to be strictly controlled, or an instrumented treadmill or several force plates in a row need to be used (Back and Clayton, 2013a).

Force plate analysis has been used in pigs to assess the effect of flooring on the gait of healthy pigs (Thorup et al., 2008, 2007; Von Wachenfelt et al., 2010; Von Wachenfelt et al., 2009; Von Wachenfelt et al., 2008). Static analysis of weight distribution between legs of standing sows has been used as well (Karriker et al., 2013; Pluym et al., 2013; Sun et al., 2011) and one of these systems is transportable, which greatly improves the usability on farms (Pluym et al., 2013).

1.3.3 Kinematics

Kinematics quantify temporal, spatial and angular gait characteristics. In order to track body segments, they need to be identified which can be done with active or passive markers, or markerless with software that recognizes parts based on pattern recognition algorithms. The markers are usually tracked with camera's that are placed around the subject in order to obtain 3D information. This means that kinematic analysis is usually limited to laboratory settings (Back and Clayton, 2013a). Markers may be placed over "landmark" anatomical bony features, however displacement of the markers by skin sliding over the bones may introduce significant error. This is particularly problematic in the proximal joints. In horses, it is to some extent possible to use correction models to account for skin movement artefacts (van Weeren et al., 1992).

Kinematic studies in pigs have been performed in research settings (Conte et al., 2015, 2014a; Stavrakakis et al., 2015a; Stavrakakis et al., 2014a, 2014b; Thorup et al., 2008, 2007), and recently efforts to perform kinematics with more accessible methods (i.e. a Microsoft Kinect® sensor) have been performed (Stavrakakis et al., 2015b). In order to use this method in practice setting or for high-throughput research applications, marker-free tracking of head movements needs to be perfected.

1.3.4 Accelerometers

Accelerometers contain small silicon beams that deform during acceleration or under the influence of gravity and that translate their deformation into voltage output. Each beam measures acceleration in 1 axis, so often they are combined to enable measurement of 3-dimensional accelerations (Back and Clayton, 2013a; Mathie et al., 2004).

Accelerometers have been extensively used in human subjects to provide information on general activity levels (Lee and Shiroma, 2014; Warren et al., 2010), as well as for more detailed analysis of movements (Mathie et al., 2004; Tao et al., 2012). Accelerometers have been used for activity monitoring, quantification of behaviour and biomechanical research in animals as well (Brown et al., 2013; Wilmers et al., 2015) In pigs, their use so far has been restricted to behaviour and activity quantification (Conte et al., 2014a; Cornou and Lundbye-Christensen, 2008; Escalante et al., 2013; Oczak et al., 2015; Pastell et al., 2013; Ringgenberg et al., 2010) and has almost exclusively been applied to sows.

1.3.5 Pressure mat analysis

Pressure mats (or pressure plates) contain a dense grid of pressure sensors that together provide the pressure profile under a surface. In gait analysis, the pressure beneath the foot is evaluated. In contrast to force plates, pressure mats can only measure force in the vertical direction. When the pressure mat is large enough to collect several footfalls, additional temporospatial data such as stance time and step length can be measured as well. This is an advantage of the pressure mat over the force plate which can only collect data on one footfall at a time (Back and Clayton, 2013a). Pressure mats have been used to quantify normal locomotion in horses (Oosterlinck et al., 2011; Oosterlinck et al., 2010a), cattle (Van Der Tol et al., 2003), sheep (Agostinho et al., 2012), cats (Lascelles et al., 2007; LeQuang et al., 2010b; Verdugo et al., 2013) and dogs (Lascelles et al., 2006; LeQuang et al., 2010a; Light et al., 2010). Additionally, lameness quantification using a pressure mat has been performed in a range of species (Carroll et al., 2008; Coetzee et al., 2014; Lascelles et al., 2010; Lequang et al., 2010; Maertens et al., 2011; Nääs et al., 2009).

In pigs, pressure mats have been used to map pressure distribution under the claw (Carvalho et al., 2009), assess the effects of an experimentally induced lameness in sows

(Karriker et al., 2013) and to assess the effect of two NSAIDs on sow lameness (Pairis-Garcia et al., 2015).

1.4: Quantifying lameness in pigs using a pressure mat

Certain requirements need to be met in order to obtain meaningful data from pressure mat parameters. First of all, the animal needs to ambulate in a straight line. Any bend in the trajectory of the animal will be reflected in the pressure profile of the limbs. Also, deviations of the head in any plane may influence weight distribution, both within and between limbs. For example, an animal that looks to the left may lean slightly to the right, thereby loading the right limbs more. This may cause an asymmetry that may be mistakenly identified as lameness. It is also important that the animal travels at a constant speed. Accelerations and decelerations may influence loading of the limbs and must therefore be avoided.

Since pigs resist being guided on a leash, the runway around the footscan is designed in such a way that the animal is guided over it in a straight line without having to be led. The sides are inclined to prevent pigs from leaning into them (See Chapter 2, Figure 1).

1.4.1 Pressure mat output

As explained above, a pressure mat is made up of many individual pressure sensors. When pressure is applied to such a sensor, pressure is translated into an electrical signal. Both the magnitude of the signal and the duration of the signal are recorded. Signals from all sensors over time are added up to construct pressure profiles and pressure curves.

Figure 1 is an example of a pressure mat recording of a pig. In the top left picture, the pressure profile of a selected footprint is shown. The pixels in the picture relate to the output of the pressure sensors, with the colors representing the magnitude of the pressure. Blue corresponds to low pressure ranges, and red to high pressure ranges. The pressure profile gives an indication of pressure distribution within a footfall. Color code is assigned to each run based on the highest and lowest pressure that is measured, so each run has its own unique relation between color code and corresponding pressure. It is therefore not possible to compare color profiles between animals or even runs.

In the bottom panel force-versus-time curves for individual footfalls are represented. Each of these curves has to be assigned to its corresponding limb. Several parameters can be derived from these curves, four of which are used consistently throughout this thesis. They will be described in more detail in the next paragraph.

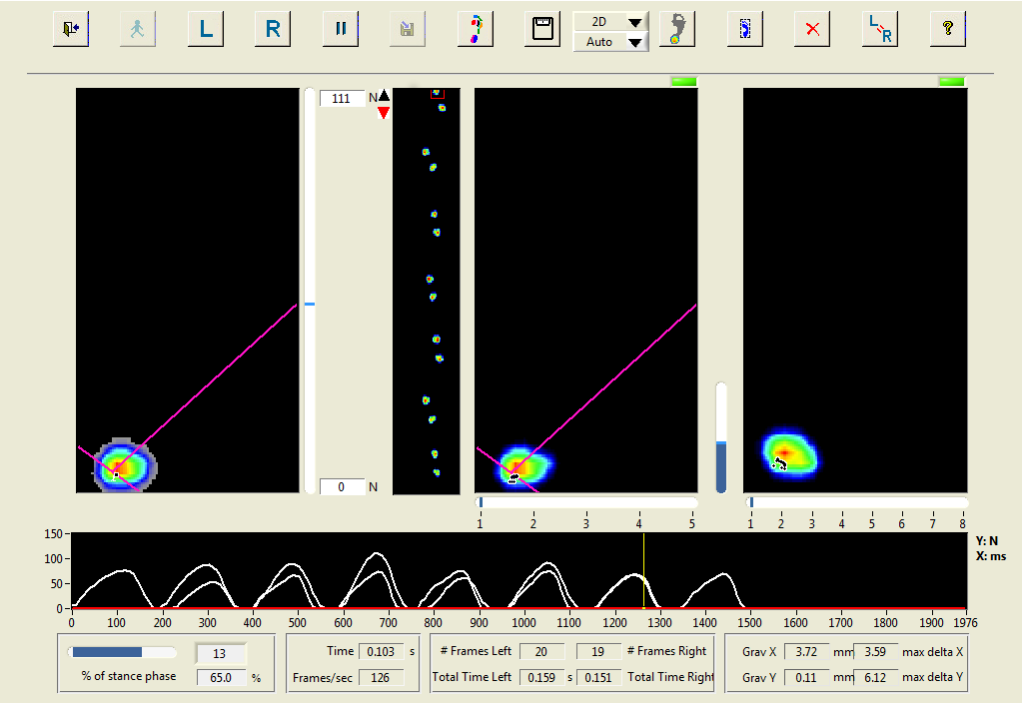

Figure 1. Typical pressure mat recording of a 10-week-old, sound pig. Color-coded pressure profiles of selected feet are shown in the top panels. The bottom panel shows force-time curves of all recorded footprints.

1.4.2 Parameters

Many parameters can be derived from pressure mat measurements. Kinetic parameters, derived from the pressure curves that are obtained, provide information on loading characteristics of limbs. They are usually descriptive on either the magnitude or the shape of the time-pressure curve. Figure 2 is a simplified example of a time-pressure curve. Four parameters are used throughout this thesis:

Peak Vertical Force (PVF) represents the highest peak in the time-force curve

Load Rate (LR) is the slope of the imaginary line from the start of the stance phase to the peak of the time-force curve

Vertical Impulse (VI) is the area under the curve

Peak Vertical Pressure (PVP) is the highest peak in the time-pressure curve, in which pressure is defined as the force divided by the contact area at that timepoint.

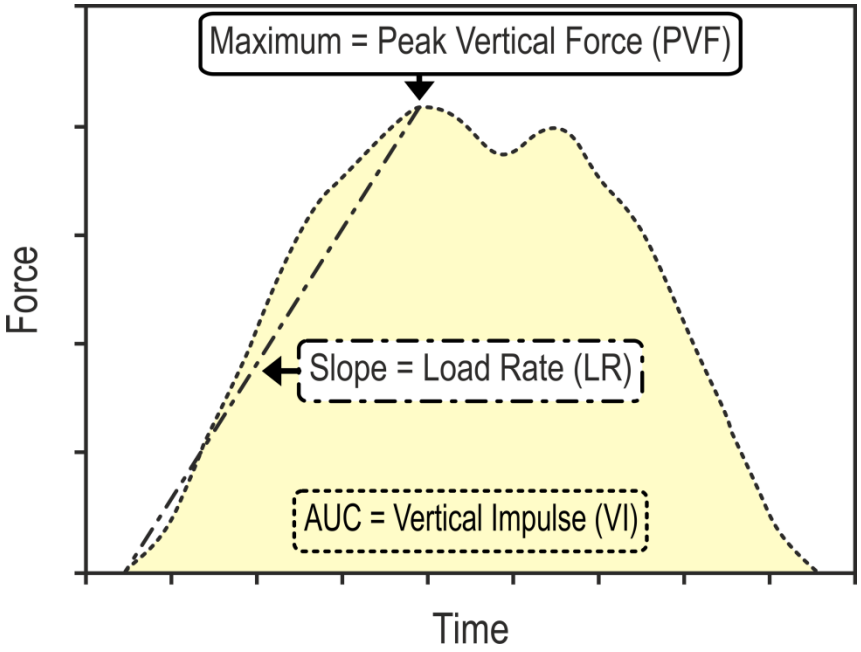

Figure 2. Schematic example of force-time curve acquired with pressure mat analysis of a walking pig. AUC=Area under curve.

1.4.3 Asymmetry as a measure of lameness

When an animal is experiencing pain in one of its limbs, it may attempt to minimize this pain by distributing its weight towards the healthy limbs, resulting in asymmetry of otherwise symmetric gaits (Abdelhadi et al., 2012; Fischer et al., 2013; Rumph et al., 1995; Weishaupt, 2008; Weishaupt et al., 2006, 2004). In kinetic as well as kinematic gait analysis, this asymmetry may be quantified by comparing parameters of healthy and affected limbs to each other using asymmetry indices.

Asymmetry indices (ASI) can be calculated in several ways. In this thesis the formula proposed by Oomen et al (Oomen et al., 2012) is used:

$$Asymmetry\ index = \frac{Left - Right}{0.5(Left + Right)} * 100$$

This formula yields a dimensionless number between -200 and +200. Positive values indicate decreased weightbearing on the right limb, and are therefore suggestive of right-limb lameness, whereas negative values suggest decreased weight-bearing on the left limb, and are therefore suggestive of left-limb lameness. A value of 0 indicates perfect symmetry.

The formula can be modified according to which limbs are compared. Figure 3 provides a schematic overview of the ASIs used in this thesis.

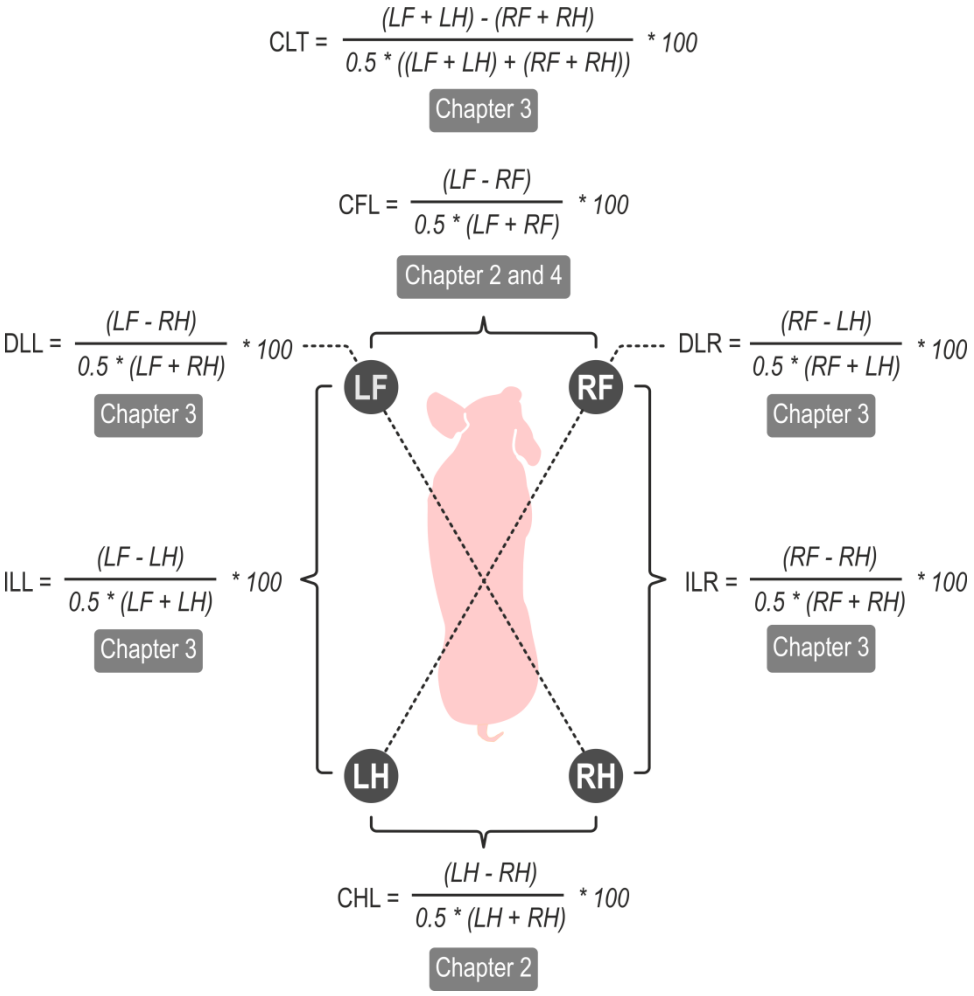

Figure 3. Formula's used to calculate asymmetry indices used in this thesis. LF=Left Front, RF=Right Front, LH=Left Hind, RH=Right Hind. CFL=Contralateral Front Limbs, CHL=Contralateral Hind Limbs, ILL=Ipsilateral Limbs Left, ILR=Ipsilateral Limbs Right, DLL=Diagonal Limbs Left, DLR=Diagonal Limbs Right.

1.5: Outline of this thesis

As described in the previous text, lameness is an important problem that affects welfare of the pig and economic profit for the farmer. In order to optimize interventions it is

important to be able to detect and quantify lameness in an objective manner. The final aim of this thesis is to evaluate pressure mat analysis as a method to quantify lameness in weaned pigs. In order to do so, the following steps were taken:

Chapter 2: Since virtually no data are available on pressure mat analysis of gait in young, growing pigs, the first experiment explored some basic concepts. The effects of several parameters on kinetic pressure mat parameters are described. A potential method to minimize the influence of these parameters and to quantify lameness, asymmetry indices, is assessed as well.

Chapter 3: The ability of the pressure mat to distinguish between clinically lame and sound pigs is assessed. Pressure mat parameters are also used to establish redistribution patterns in lame pigs.

Chapter 4: This chapter further examines the ability of the pressure mat to pick up on gait abnormalities by introducing more subtle differences in lame animals. The strong analgesic Buprenorphine is used on lame pigs, and the ability of the pressure mat to detect the effect of pain medication on lameness is evaluated. Additionally, since a decrease in activity is thought to occur in lame pigs, the effect of pain medication on exploratory behaviour in lame animals is assessed using the open field test.

Chapter 5: An example of an application of pressure mat analysis is described in this chapter as part of a multilevel pain assessment system to characterize the functional aspects of a lameness model in pigs. Furthermore, the effect of the NSAID Meloxicam on lameness due to experimentally induced osteoarthritis was assessed. This chapter also provides the possibility to compare visual scoring of subtle lameness to pressure mat analysis.

Chapter 6: The results of these experiments are discussed in the general discussion

Three appendices are added to provide some examples of related topics in lameness assessment that fall beyond the scope of this thesis.

> **Appendix 1** describes the use of temporospatial pressure mat parameters, instead of kinetic pressure mat parameters, to detect lameness in pigs
>
> **Appendix 2** is an example of pressure mat analysis in a different species, the dog.
>
> **Appendix 3** is an attempt to detect lameness in weaned pigs by another method: accelerometers

2

Pressure mat analysis of the longitudinal development of pig locomotion in growing pigs after weaning

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Abstract

Gait evaluation is difficult in pigs, especially when objective and quantitative data are needed, thus little research has been conducted in this species. There is considerable experience, however, with objective gait analysis in other species, such as horses and dogs. In this study, a pressure mat was used to establish baseline kinetic data for gait and its longitudinal development in growing, weaned piglets.

Ten clinically healthy weaned piglets were trained to trot over a pressure mat. Measurements were performed weekly during 10 weeks, starting at 5 weeks of age. Four kinetic parameters were recorded for all four limbs: peak vertical force (PVF), load rate (LR), vertical impulse (VI) and peak vertical pressure (PVP). Three representative runs per measuring session per pig were collected. For each of the variables, left vs. right limb asymmetry-indices (ASI's) were calculated based on the average for that parameter per week. A linear mixed model was used to determine the influence of time (week), velocity, and limb (left vs. right and fore vs. hind). Intra-class correlations were calculated to assess within-session replicability.

Intra-class correlations showed good within-session replicability. Body-weight normalised PVF (nPVF), LR (nLR), VI (nVI) and PVP (nPVP) were higher in the forelimbs than in the hind limbs. A higher velocity was associated with a higher nPVF, nLR and nPVP. All parameters varied between weeks. ASI of LR and VI were higher in the forelimbs than in the hind limbs. Velocity and time did not influence ASI of any of the variables.

Kinetic pressure mat measurements from healthy weaned piglets are highly replicable within-session. However, these variables present a significant variability between-session, which may be due to conformational changes of the young, growing piglets. Velocity clearly influences nPVF, nLR and nPVP, and all kinetic variables have higher values in forelimbs than in hind limbs. As time and velocity do not affect ASI's, the latter are preferable tools when velocity cannot be controlled or when measurements are repeated over longer time intervals. The present study supports the use of a pressure mat as an objective way to analyse and quantify porcine gait.

Keywords

Kinetics, Gait analysis, Pig, Symmetry

Background

Lameness is an important problem in modern swine husbandry. Prevalence of lameness in a cross-sectional study in the United Kingdom was estimated to be 14.4% in pregnant gilts, 16.9% in pregnant sows and 19.7% in finishing pigs (KilBride et al., 2009). Lameness has negative consequences from an animal welfare as well as from an economic point of view. The negative consequences of lameness on animal welfare are primarily due to pain and the resulting reduced mobility. A lame pig may not be able to reach feeding and drinking facilities and at the same time has a higher risk to be overrun by penmates, encountering additional trauma further reducing its welfare. The economic impact of lameness is caused by lower productivity and higher costs of treatment or even early culling of affected animals (S. Anil et al., 2009; Jensen et al., 2012).

To minimize the aforementioned negative consequences of lameness and increase the chances of recovery it is critical to detect lame pigs as early as possible. Subtle changes in posture or weight bearing may occur in early stages of the disease process, and can easily be missed when gait is only assessed visually (Anil et al., 2008). Furthermore, these changes can easily be overlooked in a pen with many pigs. Fast, sensitive, yet practical methods to detect lameness are necessary to help farmers to provide timely care for lame pigs, and to adequately measure the effect of interventions. Therefore, an objective method that does not only identify lame pigs but also quantifies the degree of lameness is needed to provide evidence-based information.

Several techniques have been developed for this purpose. The simplest and least expensive methods are lameness scoring systems based on visual inspection. The visual lameness scoring system for finishing pigs developed by Main et al. (2000b)incorporates gait characteristics (weight bearing on lame limb, stride length, caudal body sway), posture and behaviour (both in response to humans and within the group of animals). Although visual scoring is fast and inexpensive, research in horses and dogs has shown that it may suffer from inherent subjectivity, is affected by observer bias and has limited intra- and inter-rater agreement, especially in untrained observers and in mild lameness (Waxman et al., 2008; Arkell et al., 2006; Keegan, 2007; Quinn et al., 2007). Similarly, visual grading of mild lameness in pigs has been proven to be subjective (Anil et al., 2008). These drawbacks underline the need for a more objective method to quantify lameness.

Kinematic techniques have been used in pigs to study the effect of different flooring types on locomotion (Thorup et al., 2007; Von Wachenfelt et al., 2008) and to quantify lameness in sows (Grégoire et al., 2013), although this rather complicated, expensive and time-consuming methodology is unlikely to be extrapolated to a practical situation. Therefore,

in pigs as well as in other species, force plates have become 'the gold standard' to objectively evaluate kinetic gait variables. They have been used in pigs to study the effect of different flooring types on gait kinetics (Thorup et al., 2007) and to assess lameness in sows (Sun et al., 2011). A major drawback in the use of force plates is that they cannot distinguish between several feet simultaneously in contact with the plate. Therefore, data collection can be time-consuming, or multiple consecutive force plates are needed to gather data of all limbs. The two parameters most often used in force plate analysis, peak vertical force (PVF) and vertical impulse (VI) are strongly influenced by velocity (Khumsap et al., 2002, 2001; Riggs et al., 1993). Therefore, velocity needs to be controlled within strict limits if footfalls from different runs are to be compared to each other. This might be a problem in pigs, since they are difficult to handle and to guide over the runway at a certain pace.

Pressure mats may provide a solution to this problem, as they contain a dense array of pressure sensors with a high measuring frequency, enabling them to distinguish simultaneous impacts of different limbs. This equipment allows measuring kinetic as well as spatiotemporal data of simultaneous and even consecutive footfalls. Systems of different manufacturers have been used successfully to evaluate locomotion in sound horses (M. Oosterlinck et al., 2011; Oosterlinck et al., 2010a), cows (Van Der Tol et al., 2003), sheep (Agostinho et al., 2012), dogs (LeQuang et al., 2010a), and cats (Verdugo et al., 2013), and to assess lameness in dogs (Lequang et al., 2010; Oosterlinck et al., 2011) and cows (Maertens et al., 2011). Previous studies have shown that pressure-measuring systems may be useful to study the pressure distribution within each claw (Carvalho et al., 2009) and to measure PVF symmetry in an experimental lameness model in sows (Karriker et al., 2013). However, comprehensive baseline data describing the replicability, longitudinal development, and major confounding effects on pressure mat variables in weaned piglets are lacking.

Therefore, the aim of this experiment was to investigate the use of a pressure mat to evaluate longitudinal development of locomotion in growing, weaned pigs. Multiple pressure mat measurements were performed weekly during 10 weeks, starting at the age of 5 weeks. We evaluated the replicability of body-weight normalized peak vertical force (nPVF), load rate (nLR), vertical impulse (nVI) and peak vertical pressure (nPVP) as well as asymmetry indices (ASI's) of these variables, to establish baseline data for pressure mat analysis in growing pigs.

Results

At the start of the experiment, pigs' body mass was 6.25 ± 0.06 kg. At the end of the experiment, 10 weeks later, their body mass had increased to 34.2 ± 0.07 kg. The overall

mean velocity of valid runs was 1.53 ± 0.01 m/s. The experimental setup is illustrated in Figure 1. Bodymass, velocity and duty factor per week are summarized in Figure 2.

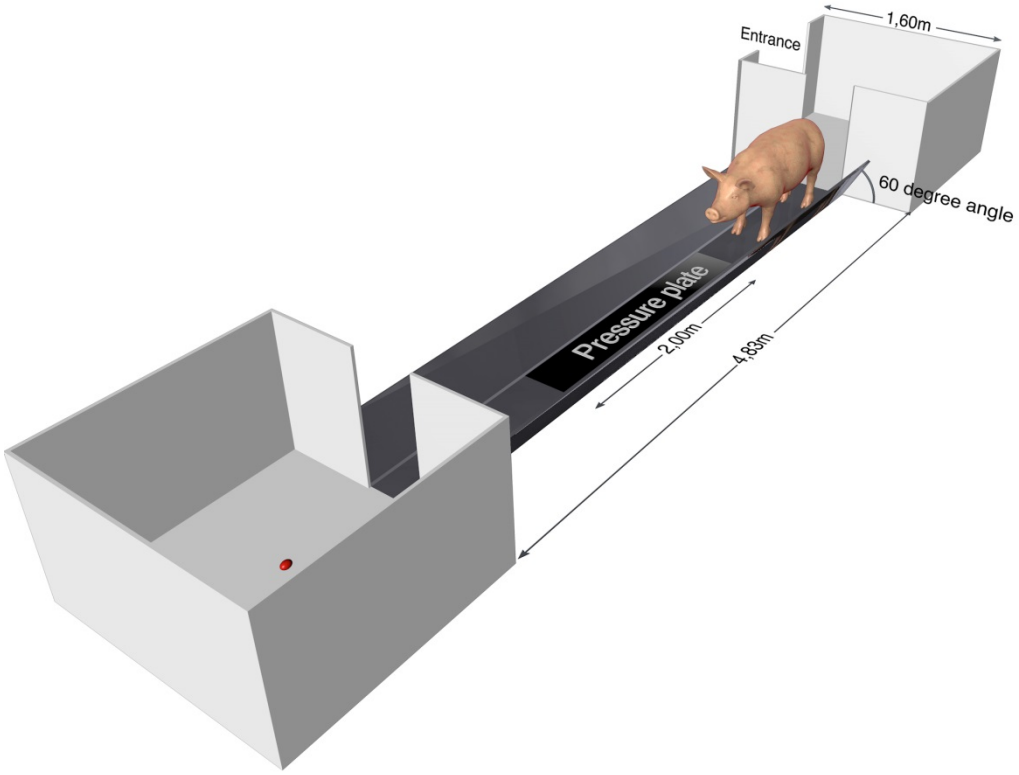

Figure 1. Schematic illustration of the experimental setup, including the runway containing a pressure mat.

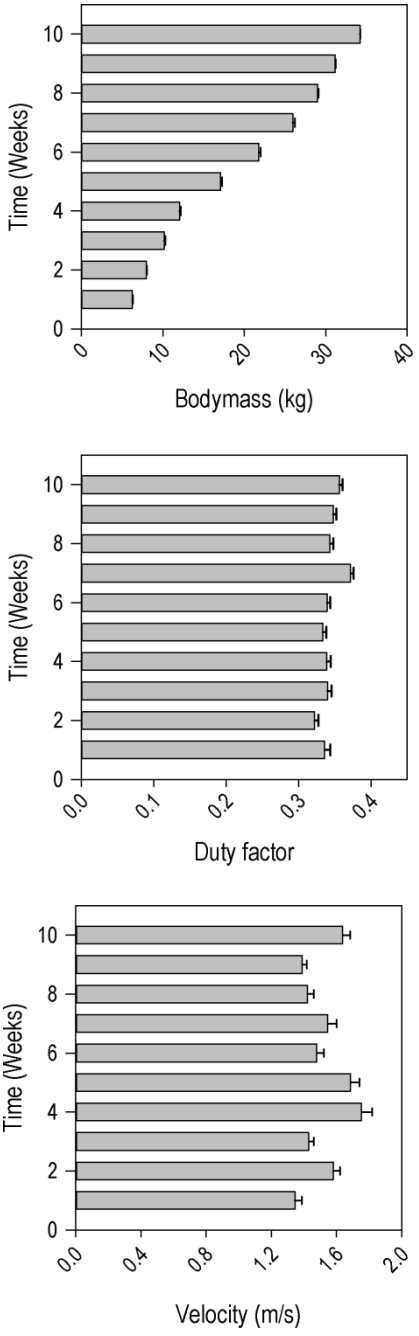

Figure 2. Velocity (mean ± SEM over all pigs, upper left panel), bodymass (mean ± SEM over all pigs, upper right panel) and duty factor (mean ± SEM over all pigs, lower left panel) for each week of the study period.

Pathology

The limb joints did not show any macroscopic abnormalities. Longitudinal cuts through the humerus, radius, ulna, femur, tibia, and fibula were macroscopically normal. No abnormalities were noted in skin, muscles, or connective tissue in any of the limbs. Therefore, all pigs were considered to be sound and healthy at scheduled necropsy.

Replicability

Intra-class correlations (ICC) between runs on the same day were fair to excellent. ICC for nLR was the lowest (0.644), followed by the ICC for nPVF (0.802) and nPVP (0.858). ICC for nVI was the highest (0.881).

Pressure mat variables

Average pressure mat variables per limb are summarized in Table 1.

Table 1. Pressure mat gait variables of trotting pigs (mean ± SEM) over all pigs and over the complete study period

Variable	Left fore	Right fore	Left hind	Right hind
Weight distribution (%)	0.29 ± 0.00	0.29 ± 0.00	0.21 ± 0.00	0.21 ± 0.00
nPVF (N/kg)	6.67 ± 0.09	6.61 ± 0.09	4.83 ± 0.09	4.93 ± 0.08
PVF (% BW)	67.99 ± 0.88	67.33 ± 0.92	49.24 ± 0.87	50.23 ± 0.78
nLR ((N/s)/kg)	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
nVI (Ns/kg	0.67 ± 0.02	0.65 ± 0.01	0.46 ± 0.01	0.47 ± 0.01
VI (s*% BM)	6.85 ± 0.15	6.65 ± 0.14	4.72 ± 0.12	4.76 ± 0.12
nPVP((N/cm2)kg)	0.31 ± 0.01	0.30 ± 0.01	0.26 ± 0.01	0.26 ± 0.00

Peak vertical force

nPVF was affected by velocity F (1, 1175) = 31.73, P < 0.05), and was different between fore vs. hind limb (F (1, 1175) = 638.07, P < 0.05), and between the different time points (F (9, 1175) = 40.27, P < 0.05), but the direction of this influence differed between weeks (Figure 3, Table 2).

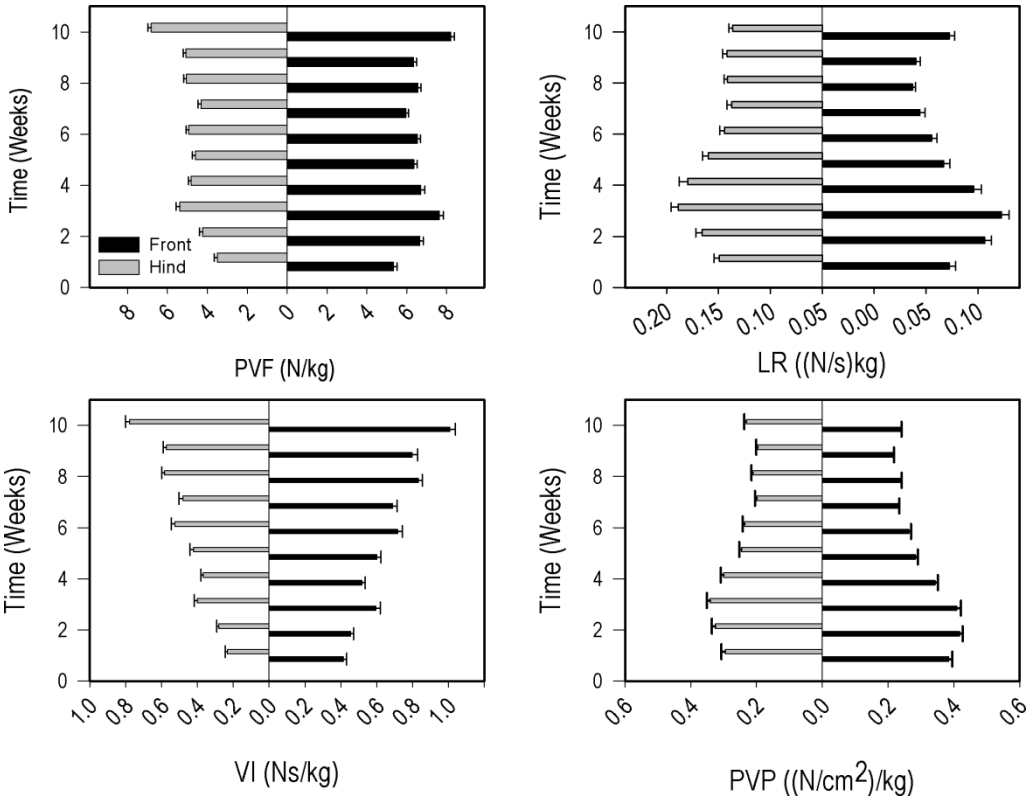

Figure 3. Fore- and hind limb pressure mat variables (mean ± SEM over all pigs) for nPVF (upper left panel), nLR (upper right panel), nVI (lower left panel) and nPVP (lower right panel) for each week of the study period. Legend in upper left panel applies to all panels. Significant differences between weeks are summarized in Tables 3, 4, 5, 6.

Table 2. Mean differences in nPVF by week controlling for velocity and fore-or hindlimb

Week	1	2	3	4	5	6	7	8	9	10
1	0.00	-0.95*	-2.05*	-1.23*	-0.96*	-1.27*	-0.66*	-1.36*	-1.27*	-3.01*
2		0.00	-1.10*	-0.28	-0.01	-0.32*	0.30	-0.41*	-0.32*	-2.05*
3			0.00	0.82*	1.09*	0.78*	1.40*	0.69*	0.78*	-0.95*
4				0.00	0.27	-0.04	0.58*	-0.13	-0.04	-1.77*
5					0.00	-0.31	0.31	-0.40*	-0.31	-2.04*
6						0.00	0.62*	-0.09	0.00	-1.73*
7							0.00	-0.71*	-0.61*	-2.35*
8								0.00	0.09	-1.64*
9									0.00	-1.73*
10										0.00

Numbers in table are column 1- row 1 difference. An * indicates a significant difference.

Load rate

nLR was higher with increasing velocity ($F(1, 1175) = 65.41$, $P < 0.05$) and was higher in forelimbs than in hind limbs ($F(1, 1175) = 25.19$, $P < 0.05$). Time affected nLR ($F(9, 1175) = 26.20$, $P < 0.05$) but the direction of this influence varied between weeks (Figure 3, Table 3).

Table 3. Mean differences in nLR by week controlling for velocity and fore-or hindlimb

Week	1	2	3	4	5	6	7	8	9	10
1	0.00	-0.02*	-0.04*	-0.02*	0.00	0.01*	0.02*	0.02*	0.02*	-0.01
2		0.00	-0.02*	0.00	0.02*	0.04*	0.05*	0.04*	0.04*	0.01*
3			0.00	0.02*	0.05*	0.06*	0.07*	0.07*	0.06*	0.03*
4				0.00	.023*	.033*	.044*	.043*	.041*	0.01
5					0.00	0.01	0.02*	0.02*	0.02*	-0.01*
6						0.00	0.01	0.01	0.01	-0.02*
7							0.00	0.00	0.00	-0.03*
8								0.00	0.00	-0.03*
9									0.00	-0.03*
10										0.00

Numbers in table are column 1- row 1 difference. An * indicates a significant difference.

Vertical impulse

Forelimb nVI was higher than hind limb nVI ($F(1, 1175) = 570.17$, $p < 0.05$) and generally increased over time ($F(9, 1175) = 71.33$, $p < 0.05$) (Figure 3, Table 4). The effect of velocity was present in the initial analysis ($p = 0.04$), but was not significant after Bonferroni correction.

Table 4. Mean differences in nVI by week controlling for velocity and fore-or hindlimb

Week	1	2	3	4	5	6	7	8	9	10
1	0.00	-0.04*	-0.17*	-0.12*	-0.19*	-0.30*	-0.26*	-0.38*	-0.36*	-0.57*
2		0.00	-0.13*	-0.07*	-0.14*	-0.25*	-0.22*	-0.34*	-0.32*	-0.53*
3			0.00	0.06*	-0.01	-0.12*	-0.09*	-0.21*	-0.19*	-0.40*
4				0.00	-0.07*	-0.18*	-0.15*	-0.27*	-0.24*	-0.45*
5					0.00	-0.11*	-0.08*	-0.20*	-0.17*	-0.38*
6						0.00	0.03	-0.09*	-0.06*	-0.27*
7							0.00	-0.12*	-0.10*	-0.31*
8								0.00	0.02	-0.19*
9									0.00	-0.21*
10										0.00

Numbers in table are column 1- row 1 difference. An * indicates a significant difference.

Peak vertical pressure

nPVP increased with higher velocity ($F(1, 1175) = 22.67$, $p < 0.05$). Forelimb nPVP was higher than hind limb nPVP ($F(1, 1175) = 164.59$, $p < 0.05$). Time influenced nPVP ($F(9, 1175) = 92.71$, $p < 0.05$), and nPVP showed a tendency to decrease over time (Figure 3, Table 5).

Table 5. Mean differences in nPVP by week controlling for velocity and fore-or hindlimb

Week	1	2	3	4	5	6	7	8	9	10
1	0.00	-0.03*	-0.03*	0.02*	0.08*	0.09*	0.13*	0.12*	0.14*	0.11*
2		0.00	-0.01	0.05*	0.11*	0.12*	0.16*	0.15*	0.16*	0.14*
3			0.00	0.06*	0.11*	0.13*	0.16*	0.15*	0.17*	0.14*
4				0.00	0.06*	0.07*	0.10*	0.09*	0.11*	0.09*
5					0.00	0.01	0.05*	0.04*	0.06*	0.03*
6						0.00	0.04*	0.03*	0.04*	0.02*
7							0.00	-0.01	0.01	-0.02*
8								0.00	0.02*	-0.01
9									0.00	-0.03*
10										0.00

Numbers in table are column 1- row 1 difference. An * indicates a significant difference.

Asymmetry indices

Average ASIsfor all parameters are summarized in Table 6. Forelimb ASIswere higher than hind limb ASIsfor nLR ($F(1,189) = 5.73$, $P < 0.05$) and nVI ($F(1,189) = 5.74$, $P < 0.05$). This difference between fore- and hind limb ASI was also present in the initial analysis of nPVF ($P = 0.03$) and nPVP ($P = 0.04$), but was not significant after Bonferroni correction. Time did not have a significant effect on any ASI (Figure 4).

Table 6. Fore and hind limb values for ASIs(mean ± SEM) for nPVF, nLR, nVI and nPVP over all pigs in the complete study period

Variable	Fore	Hind
ASI PVF	0.97 ± 1.23	-3.19 ± 1.57
ASI LR	-1.23 ± 1.60	-2.98 ± 2.27
ASI VI	2.44 ± 1.54	-2.52 ± 2.05
ASI PVP	4.02 ± 0.95	-1.92 ± 1.44

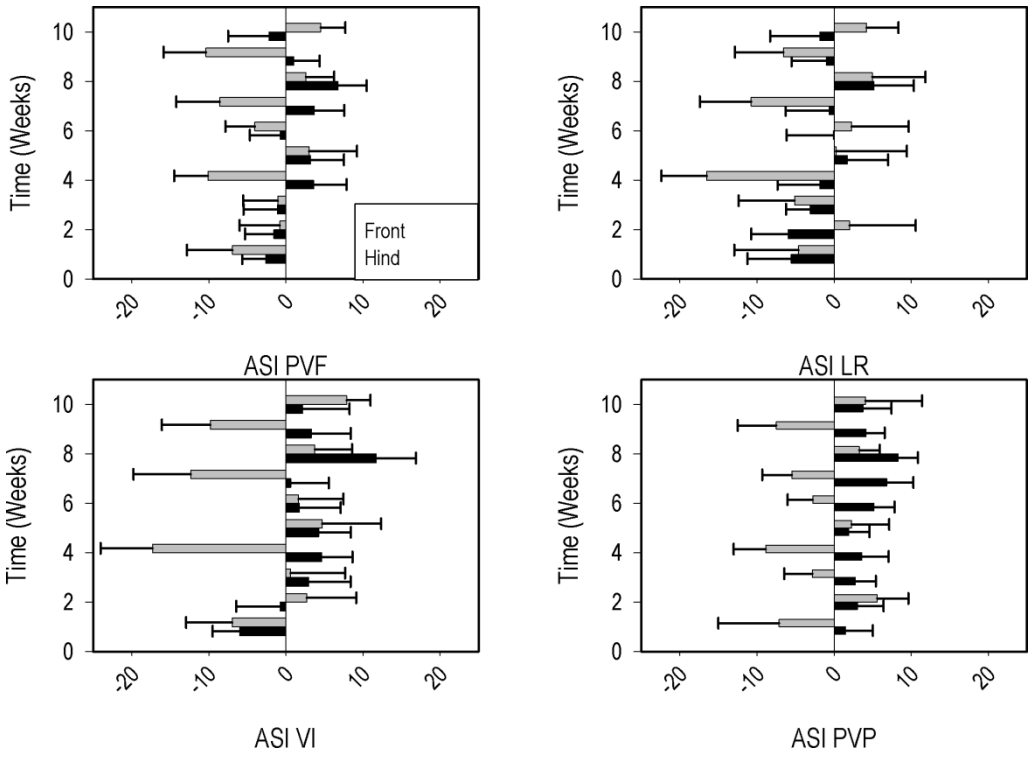

Figure 4. Absolute values for forelimb and hindlimb ASIs(mean ± SEM over all pigs) for PVF (upper left panel), LR (upper right panel), VI (lower left panel) and PVP (lower right panel) for each week of the study period. Legend in upper left panel applies to all panels.

Discussion

This study is the first to explore the use of a pressure mat for longitudinal measurement of porcine locomotion and to establish baseline data for pressure mat analysis in weaned pigs. The data collection with the pressure mat was fast and efficient, and a maximum of 10 minutes per pig was needed to collect 3 valid runs. Training of the pigs proved to be simple and effective. The preparation of data for analysis, however, was very time-consuming, mainly because the software used in this study is designed for human gait analysis, and as such could not automatically distinguish the 4 limbs of the pigs. All footprints had to be assigned manually. Some other pressure mat manufacturers (for example Tekscan ®) do provide software that can distinguish the 4 limbs of, for example dogs, automatically.

Replicability

Intra-class correlations for pressure mat kinetic variables showed that data within one day were highly replicable, which is in agreement with a previous study in ponies by Oosterlinck et al. (2010a) reporting a high replicability of nPVF and nVI, and a slightly lower replicability of nPVP. Oosterlinck et al (2010a) hypothesized that the higher variability in nPVP (which is force per unit of area) might have been due to limitations in the dynamic response of the activated sensors in the pressure mat.

Absolute values of pressure mat kinetic variables

Mass-normalized forelimb nPVF was considerably lower in trotting pigs than in trotting ponies (Barr et al., 1995; Oosterlinck et al., 2010a), horses (Oosterlinck et al., 2010b) and dogs (Lascelles et al., 2006). Mean hind limb nPVF was also lower than found in dogs (Lascelles et al., 2006). nVI of the front limbs was lower than found in dogs (Lascelles et al., 2006) and ponies (Oosterlinck et al., 2010a). Some of these differences may be associated with differences in measuring equipment setup, such as measuring frequency and calibration. The relatively low measuring frequency in our study (126 Hz) may have caused some data points to be missed. However, this explanation is unlikely considering the mean stance time of a pig in our study (176 ms). We still have an average of 22 data points per curve, resulting in a smooth force graph. Also, the calibration procedures for pressure mats vary between studies, which may make comparison of variables more difficult. When comparing pressure mat data to force plate data, it has been shown in horses that a pressure mat cannot be used interchangeably to a force plate to measure absolute values of limb loading as it has limitations in accuracy (Oosterlinck et al., 2010b), especially at impact and breakover (Oosterlinck et al., 2012). The dynamic re-

sponse of the pressure-measuring sensors may be slower than that of the piëzo-electric measurements of force plates, as was suggested by Besancon et al. (2003) and Oosterlinck et al (2010b).

The nPVP of the front limbs was higher than that found in ponies (Oosterlinck et al., 2010a). Since the nPVF was lower than in ponies, the magnitude of the nPVF cannot be the reason for the higher nPVP. Since nPVP is influenced by both nPVF and contact area, it may be that the contact properties of pig hooves promote higher nPVP. Further investigations of the nPVF and nPVP in different areas of the porcine hoof, using a pressure mat, may provide more information on this subject.

Little research is available on nLR, making this parameter hard to compare to other studies.

Effect of velocity

In this study, pigs were trained to trot across the runway at a steady pace. In studies in other species, such as dogs, horses and sheep the animals were often led by a handler. The advantage of leading the animals across the runway is that it will take less time to collect valid runs, since the animal can be guided to walk in a straight line, at a steady pace and within certain speed limits.

Since pigs resist to being handled by a collar, another method had to be used. Training the pigs to trot over the runway without any form of guidance made sure the pig was trotting in a natural pattern and looking straight ahead, not turning their head or looking up or down.

Because the pigs were running without any guidance, the velocity of the pigs could not be strictly controlled. Still, the spread of the velocities was not very large. Velocity was measured by the pressure mat. In a previous study in dogs, pressure mat and photoelectric switch measurements of velocity yielded highly similar results (Lascelles et al., 2006). Velocity significantly influenced nPVF, nLR and nPVP. The influence of velocity on nPVF is in agreement with previous reports in other species (Besancon et al., 2003; Lascelles et al., 2006; M. Oosterlinck et al., 2011; Oosterlinck et al., 2012, 2010b; Riggs et al., 1993; Roush and McLaughlin RM, 1994). In the hind limbs of walking and trotting dogs, an increase of velocity is associated with a higher nPVF and a lower nVI (McLaughlin RM and Roush, 1994; Roush and McLaughlin RM, 1994). Surprisingly, in the pigs we did not find a significant relationship between velocity and nVI. Vertical impulse is the amount of force applied over a certain amount of time (the duration of the step) and therefore depends on

the stance time and the vertical force. Normally, with increasing speed the decrease in stance time is relatively more pronounced than the increase in nPVF, resulting in a decrease in nVI. In the present study, an increase of 1 m/s in speed caused a 0.31 N/kg increase in nPVF. It is unclear whether this effect may have been large enough to outweigh the effect of decreasing stance time with speed.

There is little information on the effect of velocity on nLR in quadrupeds. McLaughlin et al (1994) did, however, show that with increasing velocity, nPVF increases and stance time decreases in both horses and dogs. This could explain the effect of velocity on load rate, since a higher nPVF has to be achieved during a shorter time period.

In the present study, contact area was not affected by velocity. As nPVP is force per unit of area, it is possible that at higher velocities nPVP increases due to increasing nPVF while contact area remains constant.

Effect of time

In this study, a significant difference between longitudinal measurements of mass-normalized nPVF, nLR, nVI and nPVP was found. It is known that besides speed, inter-trial variability is the most important confounding factor (Nordquist et al., 2011). Lascelles et al. (2006), however, did not find significant differences in pressure mat values for PVF and VI in measurements made 1 week apart in clinically normal mixed-breed dogs. Importantly, the latter studies were performed in adult dogs. In the present study, young, growing piglets were used, and even though the kinetic data were corrected for body mass, conformational changes occurred over the study period. Breed-dependent differences in kinetic data have been shown in dogs and horses (Back et al., 2007a; Mölsä et al., 2010; Voss et al., 2011). Thus, it seems possible that conformational changes in growing piglets may account for part of the longitudinal variation, especially because we followed the piglets for a long period of time compared to the studies by Mölsa et al (2010), Voss et al (2011) and Back et al (2007a).

Differences between fore- and hind limb

Our results on the difference in fore- and hind limb data for nPVF are in agreement with data in other species with a reported distribution of bodyweight of approximately 30% on each front limb and 20% on each hind limb (Besancon et al., 2003; Budsberg et al., 1987; Kim and Breur, 2008).

Asymmetry indices

Symmetry is generally assumed to be a characteristic feature of normal locomotion (Besancon et al., 2004; Budsberg et al., 1993; Herzog et al., 1989; Jeleń et al., 2008; Oosterlinck et al., 2010a), whereas a substantial lack of symmetry usually correlates with the presence of pathology/lameness (Fanchon and Grandjean, 2007; Lequang et al., 2010; Oosterlinck et al., 2011). The trot is a symmetric gait, facilitating comparison of left vs. right limbs. Our results indicate a very low degree of asymmetry in sound pigs. However, some degree of asymmetry can even be observed in sound individuals (Colborne et al., 2008; Oosterlinck et al., 2010a). Similar as reported in dogs (Oosterlinck et al., 2011), the 2 m-pressure mat in the present study allowed the recording of contra-lateral and consecutive foot strikes, and therefore, ASI between contra-lateral limbs were not affected by inter-trial variability. Notwithstanding the fact that the range of ASI observed in the present study was larger than the degree of (a)symmetry reported in dogs (Oosterlinck et al., 2011) and ponies (Oosterlinck et al., 2010a), there were no significant differences in ASIs over a prolonged time. Therefore, ASIs are a highly promising tool for the longitudinal analysis of locomotion, prospecting evidence-based evaluation of lameness, effects of treatments etc. Cut-off values of ASI obtained from pressure mat analysis to distinguish lame and sound pigs have not yet been determined, but the present study provides normative data for ASIsin young, sound pigs.

In agreement with previous work by Oosterlinck et al (Oosterlinck et al., 2010a), our results did not present a significant influence of velocity on ASIs. This is particularly interesting for the measurement of gait in pigs, since in this species it is difficult to maintain a fixed speed over several trials without disturbing the natural gait of the animal. From a practical point of view, for this type of pressure mat further development of software for use in quadrupeds is needed. In order to facilitate the analysis and subsequent interpretation of kinetic symmetry in a clinical situation using this particular kind of pressure mat, automated selection and calculation of symmetry ratios would be interesting. The software currently available allows the automated selection of human feet, whereas in our study, manual selection of each footprint was needed. In large datasets, this may be time consuming and therefore the automated allocation of left/right fore and hind hoof prints in combination with automated calculation of (a-)symmetry indices between contra-lateral, ipsilateral and diagonal limb pairs would facilitate a swift interpretation of kinetic data.

Conclusions

The present study provides normative kinetic data for young, sound pigs. Based on the significant effects of velocity, fore vs. hind limb, and measuring session on absolute values of kinetic variables, it is advised to set limits for speed. Moreover, measurements that are set apart in time (e.g. intervention studies) should be interpreted cautiously, especially in young growing pigs in which conformation may change. Fore- and hind limbs present different absolute values of limb loading and this must be accounted for when interpreting results.

In the present study, the pressure mat allowed recording contra-lateral and consecutive foot strikes. ASIs of contra-lateral limbs were shown to have excellent replicability over time, and were not affected by speed. Therefore, we recommend the use of ASIs of kinetic variables in further studies focusing on the discrimination between lame and sound pigs, the early detection of lameness, and the evidence-base evaluation of treatments.

Methods

The study was reviewed and approved by the ethics committee of Utrecht University, The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

Animals

Ten 4-week-old healthy and sound Topigs 20 pigs (6 boars, 4 sows) were randomly selected from a commercial breeding farm. The pigs were transported to the animal facility of the Veterinary Faculty, Department of Farm Animal Health, Utrecht University.

Housing

The pigs were housed in the research facility of Utrecht University. They were randomly divided over two pens with closed concrete floors, each pen measuring 153 cm × 256 cm. The ambient temperature in the stalls was 24 °C. Two extra heat lamps per pen were provided during the first 6 weeks of the experiment. The piglets were exposed to both daylight and artificial lighting from 7 a.m. to 6 p.m. (11 hours a day). They had ad libitum access to water and food (Groeiporco, De Heus Animal Nutrition, Ede, The Netherlands). The pens were provided with toys (metal chain, plastic ball) during the entire experiment.

Data recording

Every week, body mass was recorded using a weighing scale (MS Schippers, Bladel, The Netherlands), they were visually evaluated for lameness by a veterinarian using a scoring system modified from De Koning et al. (2012), and pressure mat analysis was performed. The pressure mat was a Footscan® 3D Gait Scientific 2 m system (RSscan International, Olen, Belgium) with an active sensor surface of 1.95 m × 0.32 m containing 16384 sensors (2.6 sensors per cm^2), with a sensitivity of 0.27-127 n/cm^2 and a measuring frequency of 126 Hz, connected to a laptop with dedicated software (Footscan Scientific Gait 7 gait 2nd generation, RSscan International, Olen, Belgium). Calibration of the pressure mat was performed according to the manufacturer's instructions using a person weighing 70 kg. The mat was mounted flush with a 483 cm × 40 cm walkway. The entire walkway was covered with a 0.5 mm rubber mat (shore value 65° ± 5). To prevent the pigs from leaning against the wall and inadvertently influencing the measurements, the sides of the runway were inclining outward in a 60° angle. A 160 cm × 150 cm holding pen that could be closed was located at both ends of the runway (Figure 1). Velocity was measured by the pressure plate.

Procedure

After the piglets arrived at the facility, they were allowed to acclimatize to the new environment for one week. On day 1, 3, and 5 of this first week, the pigs were habituated to the test apparatus and trained to trot over the pressure mat, using treats as reward when the animal had trotted over the runway without stopping. The training ended after the piglet had performed 3 correct runs. A training session was never longer than 10 minutes, so even after multiple unsuccessful attempts, after 10 minutes the piglets were returned to their pen. It took 2-3 training sessions to train the desired behaviour in all pigs.

After the acclimatization period, measurements were started at 5 weeks of age. After 10 weeks the pigs were euthanized. The piglets were sedated using a 2 mg/kg intramuscular injection of Azaperone (Stresnil, Elanco Animal Health, Greenfield, USA). When the piglets were sufficiently sedated (no reaction to touch) they were euthanized by intracardial injection of 200 mg/kg Pentobarbital (Euthanimal, Alfasan, Woerden, The Netherlands). After euthanasia, the piglets were transported to the Department of Pathobiology of the Faculty of Veterinary Medicine of Utrecht University. Gross pathology was performed to confirm that the piglets were healthy at the time of death. Moreover, specific attention was paid to the limb joints. They were opened and inspected for any macroscopic signs of joint disease.

Pressure mat analysis

Pigs were tested in the order they presented themselves, to minimize handling-associated stress. This appeared to be a highly stable order over the study weeks.

To perform the pressure mat analysis, the pigs were individually let out of their pen. They walked freely to the testing area. When they entered the holding pen, the area was closed and testing started. Two researchers, one in each of the holding pens, rewarded the pig only when it performed a correct run (crossing the entire length of the runway at the trot without stopping). If the run was not correct, the pig received no reward. If the pig still had not performed a correct run after 10 trials, it was placed back in the home pen and tested again one hour later. This occurred only once during the complete experiment.

A correct run had to fulfill the following additional criteria to be considered valid and to be included in the study: the pig had to trot the entire length of the runway at a visually steady pace in a straight line and looking straight ahead. These criteria were judged by two observers. At least 3 valid runs per pig were collected. Velocity was recorded by the pressure mat. All analised variables were automatically generated by the software.

Data analysis

Claw strikes from the 3 valid runs were manually assigned to left fore (LF), right fore (RF), left hind (LH) and right hind (RH) limb. PVF (N), LR (N/s), VI (Ns) and PVP (N/cm^2) were normalized to body mass (nPVF, nLR, nVI, nPVP). For every pig, mean nPVF, nLR, nVI and nPVP were calculated for each set of 3 valid runs. To allow comparison with data published by others (Back et al., 2007a; Barr et al., 1995; Besancon et al., 2003; Budsberg et al., 1987; Kim and Breur, 2008), PVF and VI were also represented as percentage of bodyweight (% BW). For each run, the PVF was used to calculate the distribution of bodyweight over the four legs using the following formula:

$$\frac{\text{PVF of the limb}}{\text{total PVF of the four limbs}} * 100$$

Fore and hind limb asymmetry indices (ASI) of all variables were calculated using the following formula (Oomen et al., 2012):

$$\frac{L-R}{0.5(L+R)} * 100$$

According to this method, a value of 0% indicates perfect contra-lateral symmetry, whereas positive or negative values indicate relatively higher loading of the left or right limb, respectively. Possible values range from -200% to 200%.

For further statistical analysis, the absolute value of the ASIs was used, removing the distinction between right- or left-sided asymmetry.

Statistics

A linear mixed effects model was used to evaluate the effect of week, limb (left vs. right and fore vs. hind) as fixed factors and velocity as covariate on nPVF, nLR, nVI, nPVP and their ASI's. nLR, nVI and nPVP were log-transformed, and square root transformation of ASIs was used to meet normality assumptions. Data were analised using SPSS statistics 20 (IBM) and R 2.15 (R foundation for statistical computing) with Bonferroni-corrected statistical significance set at $p < 0.05$. In order to assess variability between runs of a pig on the same day, intra-class correlations (ICC) were calculated and interpreted according to Shrout and Fleiss (1979). The data are presented as means ± standard error of mean (SEM).

Competing interests

The authors declare that they have no financial or non-financial competing interests.

Authors' contributions

EM contributed to study design and data collection, analised the data and drafted the manuscript. CB contributed to study design and data collection. MO provided advice on pressure mat data collection and analysis, and critically revised the manuscript. FJS aided in writing the first draft of the manuscript, provided advice on data analysis and critically revised the manuscript. WB contributed to study design, provided advice on automated gait analysis and critically revised the manuscript. AvN critically revised the manuscript. All authors read and approved the final manuscript.

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3

Pressure mat analysis of naturally occurring lameness in young pigs after weaning

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Abstract

Lameness is a common problem in modern swine husbandry. It causes welfare problems in affected pigs as well as financial problems for farmers. To minimize these negative consequences of lameness, new treatment and prevention strategies need to be developed and validated using objective and quantitative measurement techniques. An example of such a putative diagnostic tool is the use of a pressure mat. Pressure mats are able to provide both objective loading (kinetic) as well as objective movement (kinematic) information on pig locomotion.

In this study, pressure mat analysis was used to assess compensatory force redistribution in lame pigs; in particular a predefined set of four pressure mat parameters was evaluated for its use to objectively distinguish clinically lame from sound pigs. Kinetic data from 10 clinically lame and 10 healthy weaned piglets were collected. These data were analysed to answer three research questions. Firstly the pattern of compensatory weight distribution in lame animals was studied using the asymmetry indices (ASI) for several combinations of limbs. Secondly, the correlation between total left-right asymmetry index and visual scores of lameness was assessed. Thirdly, by using receiver-operated curve (ROC) analysis, optimal cutoff values for these ASIs were then calculated to objectively detect lame pigs.

Lame animals generally showed a shift in loading towards their diagonal and contralateral limbs, resulting in a clear left-right asymmetry. The degree of lameness as graded by visual scoring correlated well with the total left-right ASIs. Lame pigs could be objectively distinguished from sound pigs based on clear cutoff points calculated by ROC analysis for the complete set of four evaluated parameters.

The gait of lame pigs is asymmetric, due to the unloading of the affected limb and concomitant weight redistribution towards other limbs. This asymmetry, objectively expressed as total left-right asymmetry, correlates well with the subjective visual lameness scoring and can be used to objectively distinguish lame from sound pigs. Pressure mat gait analysis of pigs, therefore, appears to be a promising and useful tool to objectively quantify and possibly early detect lameness in pigs.

Keywords

Kinetics, Gait analysis, Porcine, Symmetry, Redistribution, Loading

Background

Lameness in pigs is a common problem in modern swine husbandry, affecting up to 19% of finishing pigs (KilBride et al., 2009). It has negative consequences both from an animal welfare as well as from an economic point of view. Lameness seriously impairs welfare and may have an effect on all of Brambell's "five freedoms" (Anil et al., 2009). The economic impact of lameness is caused by lower productivity in lame animals, the cost of treating affected animals and the cost of premature culling of animals (Anil et al., 2005; Jensen et al., 2012). The aforementioned welfare and economic issues can be minimized if veterinarians provide evidence-based advice on treating and preventing lameness. Detection of lameness needs to be sensitive and reliable, and treatment and prevention strategies need to be assessed using objective, repeatable methods to quantify lameness.

As already proven in other animals including man, pressure mats are a noninvasive and objective tool to quantitatively assess gait, providing kinetic and temporospatial data. Their use has increased lately as they seem to have some distinct advantages over other, 'classical' methods to analyse gait, like force plates and infrared high-speed camera systems. When using pressure mats, it is possible to measure consecutive, overlapping and even simultaneous footfalls, thus enabling the collection of several footfalls in one run. Moreover, an elaborate calibration setup that some other, aforementioned methods would require for a 3D kinetic and kinematic analysis is not needed. In addition, pressure plates with high sensor density provide information on the pressure distribution between different regions, for example between different claws within the foot.

Pressure mats have been used to study gait in several other quadruped species, such as horses (Oosterlinck et al., 2010a), cattle (Van Der Tol et al., 2003), sheep (Agostinho et al., 2012), dogs (Lequang et al., 2010), and cats (Verdugo et al., 2013). Most of these studies focused on sound animals. Considerably less information, however, is available on the sensitivity and reliability of pressure mats to objectively distinguish lame from sound animals. Earlier studies in dogs and cattle indeed reported that it is possible to distinguish lame from sound individuals (Fanchon and Grandjean, 2007; Lequang et al., 2010; Maertens et al., 2011; Oosterlinck et al., 2011). However, hardly any information on the use of pressure mat analysis to detect lameness in pigs is available yet. Only one single study was identified that described lameness in sows using pressure mat analysis, but only peak vertical pressure symmetry was used to objectively quantify lameness (Karriker et al., 2013).

Therefore, the objective of this study was to evaluate the ability of four kinetic pressure mat parameters (peak vertical force: PVF, load rate: LR, vertical impulse: VI, and peak vertical pressure: PVP) to distinguish clinically lame from sound weaned piglets.

We assessed compensatory weight redistribution by calculating the asymmetry-indices (ASI) for several combinations of limbs and for each of the pressure mat parameters. In order to compare this new method to the method that is currently used most often in lameness research in pigs (visual scoring), correlation of total left-right ASIs for the four pressure mat parameters with visual scores of lameness were assessed as well. We used receiver-operated characteristic (ROC) curve analysis to determine the optimal cutoff values of total left-right asymmetry ASI to identify lame pigs.

Results

Mean body mass was 9.5 ± 2.0 kg for the lame pigs and 9.1 ± 1.7 kg for the sound pigs and did not differ between groups ($t(18) = 0.462$, $p = 0.650$). Mean velocity was 1.3 ± 0.5 m/s in the sound pigs and 0.6 ± 0.4 m/s in the lame pigs. The velocity was lower in the lame pigs compared to the sound pigs ($t(18) = 4.146$, $p = 0.001$).

The visual gait score (0 = sound, 5 = non-weigh bearing lameness) in the lame pigs ranged from 2 to 4 with a mean of 3.0 ± 0.8.

Details of the gross pathological examination and the visual lameness scoring of the lame animals are shown in Table 1.

Pressure mat analysis yielded prints for each claw (See Figure 1 for an example of pressure mat recordings).

Table 1: Gender, weight, diagnosis and localization of lameness in lame pigs

ID no.	Gender	Body mass (kg)	Lame limb	Diagnosis	Location	Visual lameness score
2456	F	10.3	RF	Periarthritis	Carpus	2
5685	M	13	RH	Periarthritis + Arthritis	Tarsus	4
4155	M	7.4	LH	Arthritis	Metatarsophalangeal joint	3
2602	M	10.7	RH	Periarthritis	Distal interphalangeal joint	2
6744	M	8.6	LH	Periarthritis	Tarsus + knee	2
9559	F	7.8	RF	Periarthritis + Arthritis	Metacarpophalangeal joint	3
7173	M	8.5	RF	Periarthritis	Elbow	4
2879	F	9.7	RH	Periarthritis + Arthritis	Tarsus	3
2408	F	12.1	LH	Arthritis	Metatarsophalangeal joint	4
4751	M	7.2	LF	Periarthritis	Carpus	3

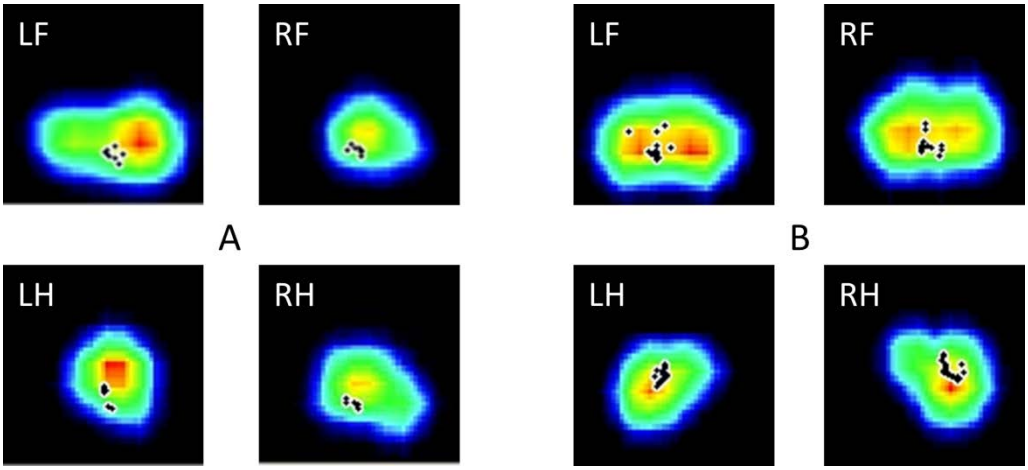

Figure 1. Typical pressure mat recordings from pig with right forelimb lameness (A) and sound pig (B). LF = left front, RF = right front, LH = left hind, RH = right hind. Pig a had a visual lameness score of 2/5. The recorded pressure is represented by colour: red for the highest pressure, blue for the lowest pressure. Black dots represent the centre of pressure throughout the stance phase. Pig A is showing a clear difference in the amount of pressure applied between the lame foot (RF) and the contralateral foot (LF), while also showing pressure redistribution toward the left hind limb.

Redistribution of pressure

Figures 2 to 7 present the ASIs of three groups of animals: sound animals, animals that were lame on a limb that was assessed by that particular ASI and animals that were lame on a limb that was not assessed by that ASI. Full test statistics and exact p-values are provided as Additional file 1.

Contralateral forelimb ASI for PVF differed between groups ($\chi^2(2, N = 20) = 11.61$, $p = 0.003$) and was higher in the animals that were lame on a forelimb compared to animals that were lame on a hind limb or animals that were not lame (see Figure 2). Load rate CFL differed between groups as well ($\chi^2(2, N = 20) = 11.09$, $p = 0.004$) and animals that were lame on a forelimb had higher CFL than animals that were lame on a hind limb and sound animals. A difference between all groups was present in the CFL of VI ($\chi^2(2, N = 20) = 12.19$, $p = 0.002$). Although CFL of PVP also differed between groups ($\chi^2(2, N = 20) = 7.01$, $p = 0.030$), the Mann-Whitney U test only showed a difference between sound animals and animals that were lame on a forelimb.

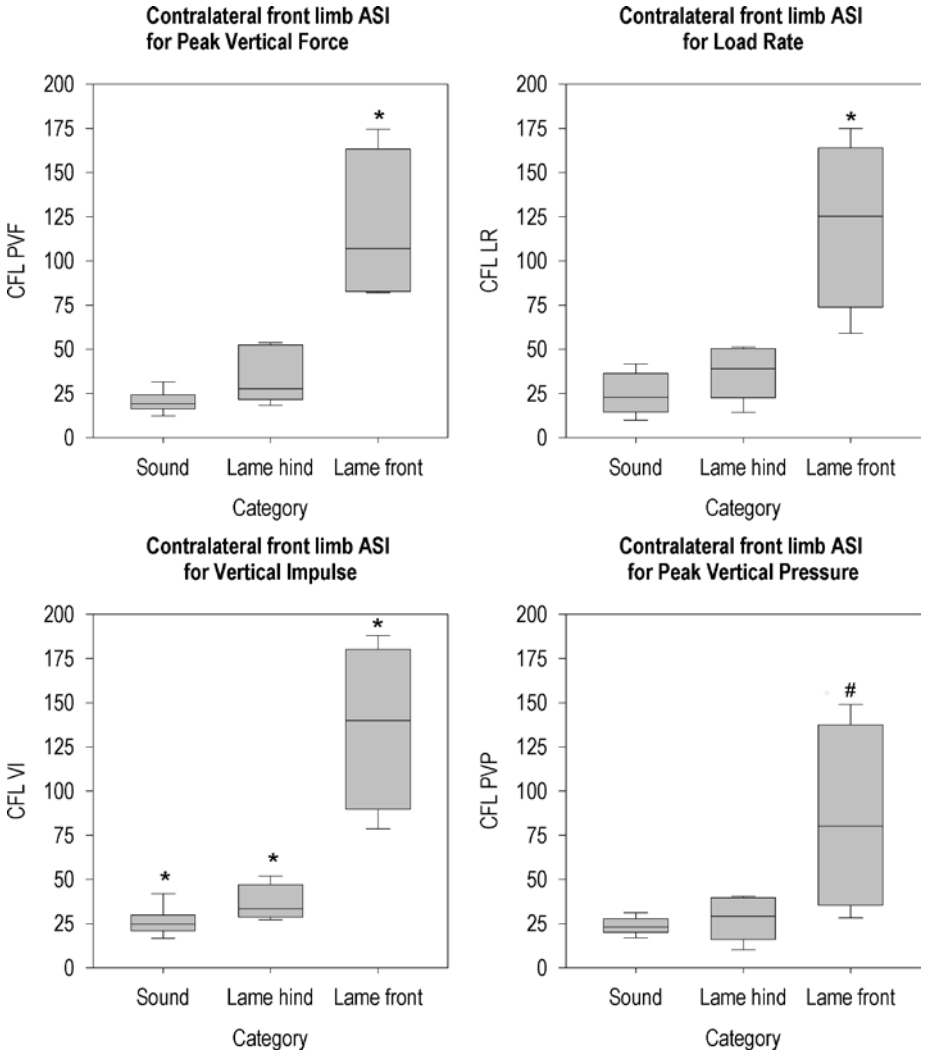

Figure 2. Contralateral forelimb ASI per group for all parameters. * The ASI of this group differs from the ASIs of the other two groups ($p < 0.05$). # The ASI of this group differs from the sound group ($p < 0.05$).

Contralateral hind limb ASI (Figure 3) was different between groups for all parameters (PVF ($\chi^2(2, N = 20) = 15.48$, $p = 0.000$), LR ($\chi^2(2, N = 20) = 14.58$, $p = 0.001$), VI ($\chi^2(2, N = 20) = 15.48$, $p = 0.000$) and PVP ($\chi^2(2, N = 20) = 14.29$, $p = 0.001$)). The posthoc Mann-Whitney U test showed a difference between all groups for PVF and VI. Sound animals had lower ASIs than animals that were lame on a forelimb or animals that were lame on a hind limb for LR and PVP, but there was no difference between animals that were lame on a forelimb and animals that were lame on a hind limb for these parameters.

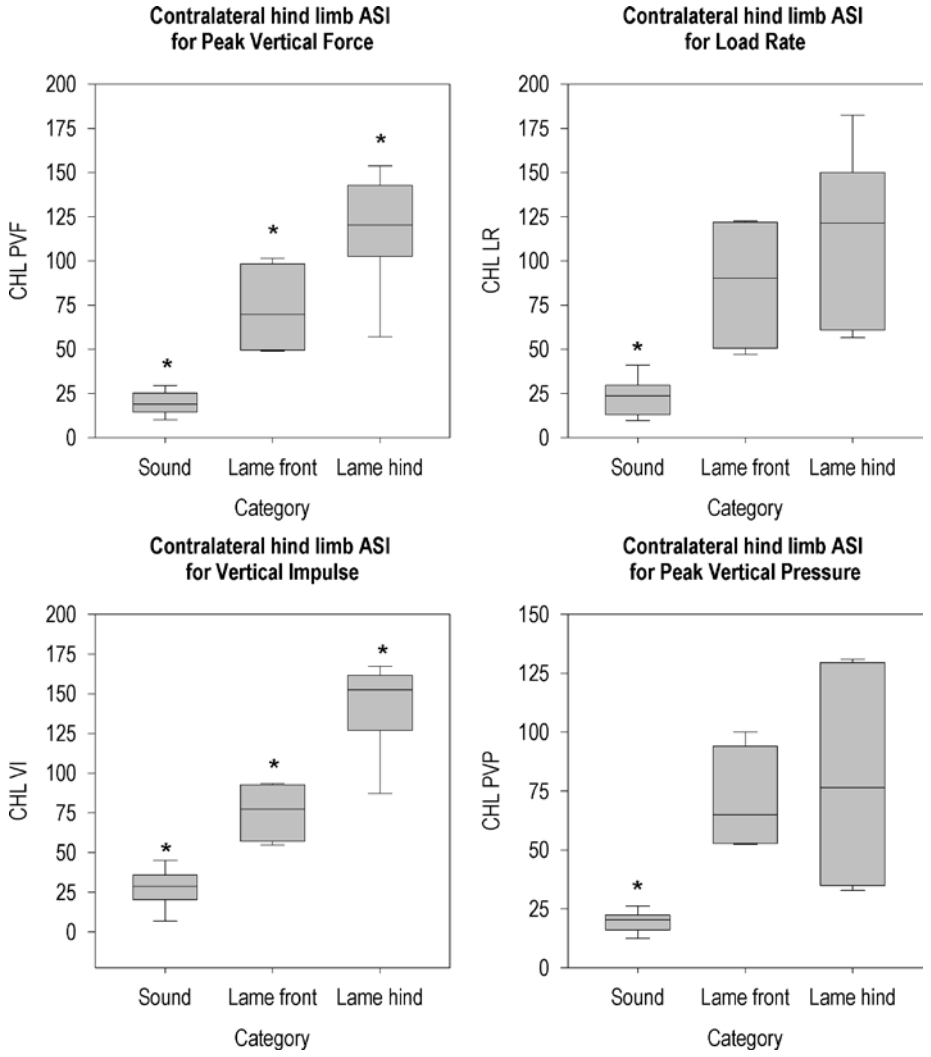

Figure 3. Contralateral hind limb ASI per group for all parameters. * The ASI of this group differs from the ASIs of the other two groups ($P < 0.05$).

The ASI of the ipsilateral left limbs (Figure 4) differed between the three groups for PVF ($\chi^2(2, N = 20) = 11.09$, $p = 0.004$), LR ($\chi^2(2, N = 20) = 9.07$, $p = 0.011$) and VI ($\chi^2(2, N = 20) = 8.73$, $p = 0.013$), but not for PVP. In the post-hoc analysis of ILL of PVF, pigs that were lame on the left limb had higher ILLs than pigs that were lame on the right limb and sound pigs. ILL of LR and VI were higher in pigs that were lame on the left limb compared to sound animals.

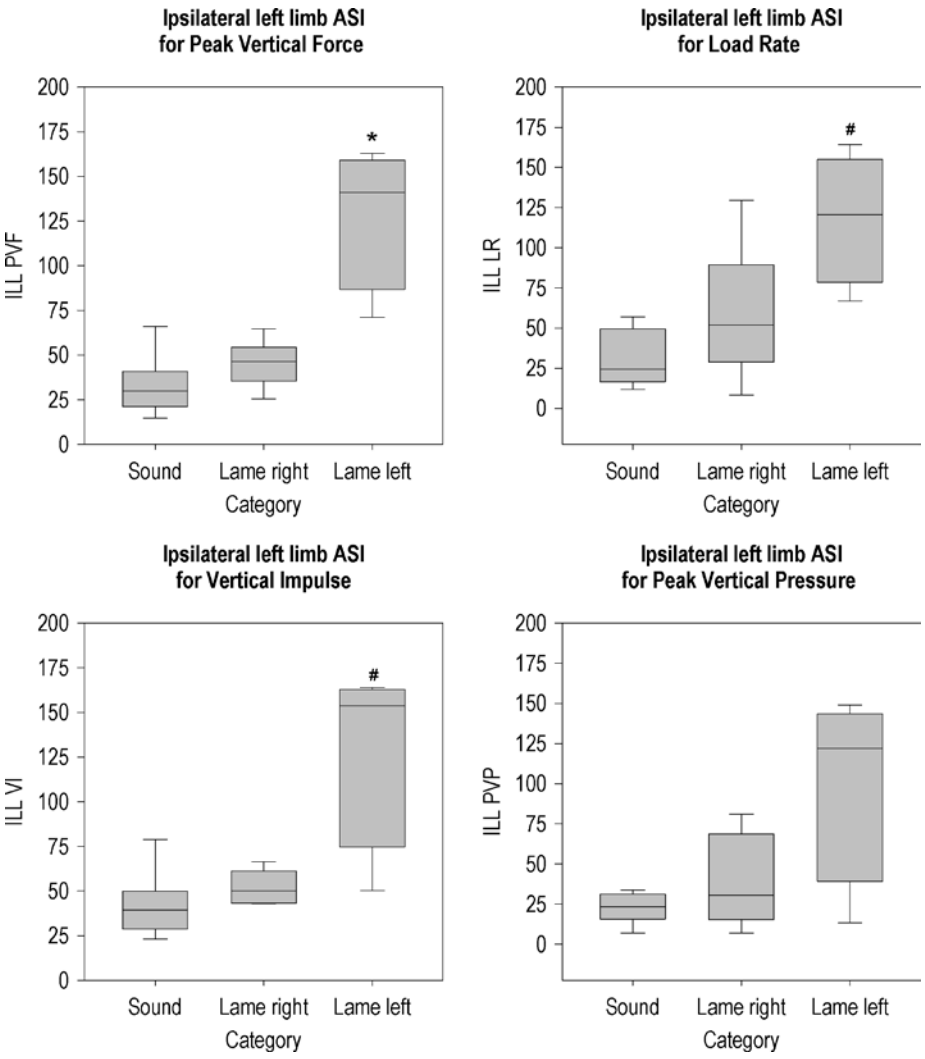

Figure 4. Ipsilateral left limb ASI per group for all parameters. * The ASI of this group differs from the ASIs of the other two groups ($P < 0.05$). # The ASI of this group differs from the sound group ($P < 0.05$).

Ipsilateral right limb ASI (Figure 5) differed between groups for PVF ($\chi^2(2, N = 20) = 6.32$, $p = 0.042$) and PVP ($\chi^2(2, N = 20) = 9.53$, $p = 0.009$), and the posthoc Mann-Whitney U test showed the animals that were lame on a right limb had higher ASIs than sound animals. The difference between groups was also found for LR ($\chi^2(2, N = 20) = 8.22$, $p = 0.016$), with sound animals having lower ASIs than lame animals, regardless of which limb was lame. VI ($\chi^2(2, N = 20) = 7.84$, $p = 0.020$) differed between groups with the animals that were lame on the right limbs having higher ASIs than other animals.

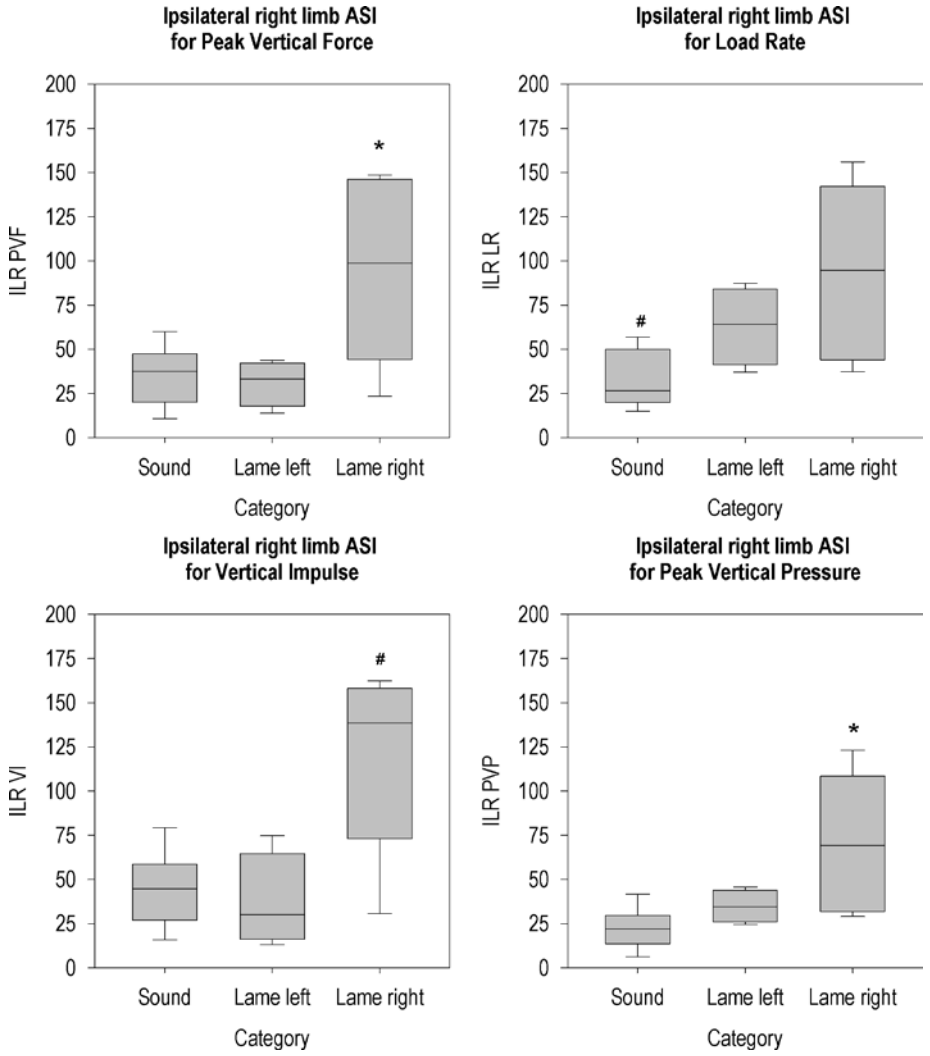

Figure 5. Ipsilateral right limb ASI per group for all parameters. * The ASI of this group differs from the sound group ($P < 0.05$). # The ASI of this group differs from the ASIs of the other two groups ($P < 0.05$).

For all parameters, diagonal LF/RH ASI (Figure 6) was different between groups (PVF ($\chi^2(2, N = 20) = 9.52$, $p = 0.009$), LR ($\chi^2(2, N = 20) = 10.94$, $p = 0.004$), VI ($\chi^2(2, N = 20) = 9.15$, $p = 0.010$), PVP ($\chi^2(2, N = 20) = 13.91$, $p = 0.001$)). Animals that were lame on the left fore or right hind limb had higher DLL than animals than animals that were lame on the right fore or left hind limb and had higher DLL than sound animals for PVF and VI. LR DLL was lower in sound animals than in other animals. For PVP, all groups differed from each other, with animals that were lame on the left fore or right hind limb having higher DLL than animals that were lame on the right fore or left hind limb and sound animals.

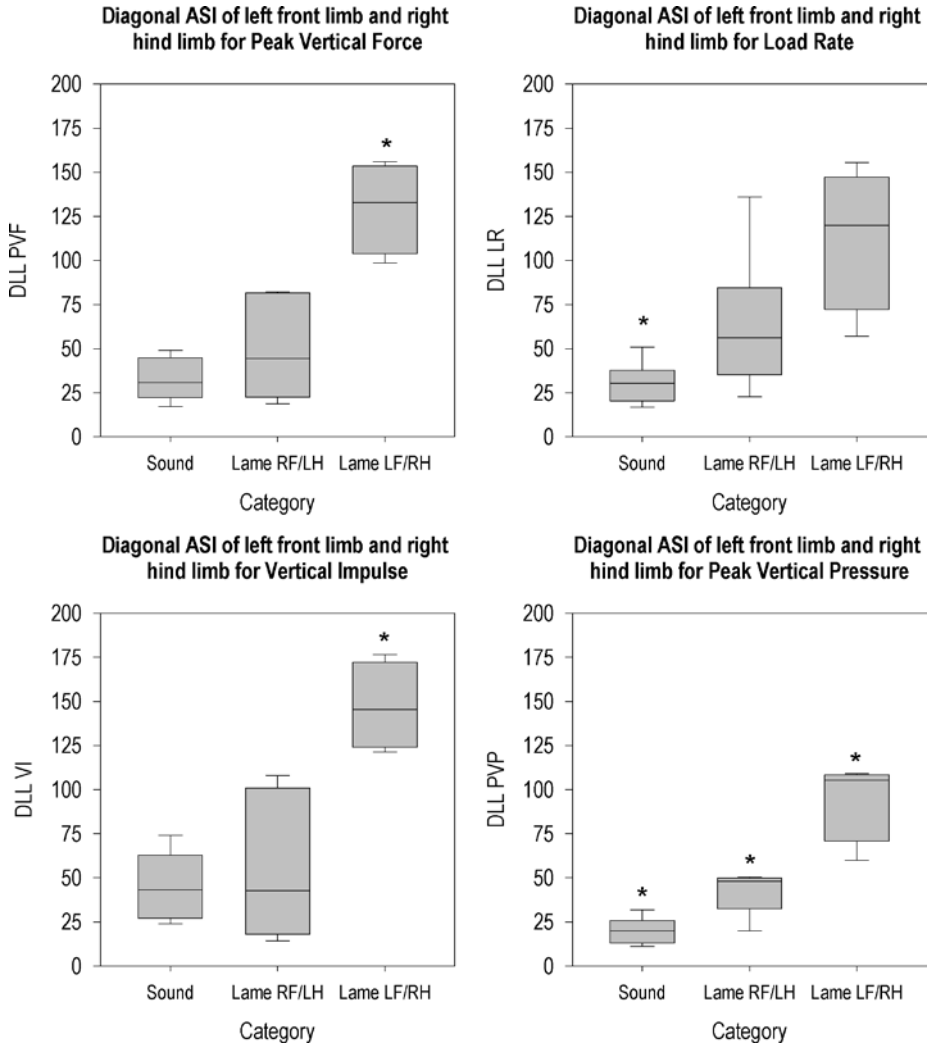

Figure 6. Diagonal ASI for left forelimb and right hind limb per group for all parameters. * The ASI of this group differs from the ASIs of the other two groups ($P < 0.05$).

Diagonal ASI of right front limb and left hind limb (Figure 7) differed between groups for all parameters (PVF ($\chi^2(2, N = 20) = 9.14$, $p = 0.010$), LR ($\chi^2(2, N = 20) = 14.66$, $p = 0.001$), VI ($\chi^2(2, N = 20) = 9.33$, $p = 0.009$), PVP ($\chi^2(2, N = 20) = 13.01$, $p = 0.001$)). Pigs that were lame on the right fore or left hind limb had higher DLR for PVF and VI compared to the sound group. For LR, all groups differed from each other. Sound animals had lower PVP DLR than lame animals.

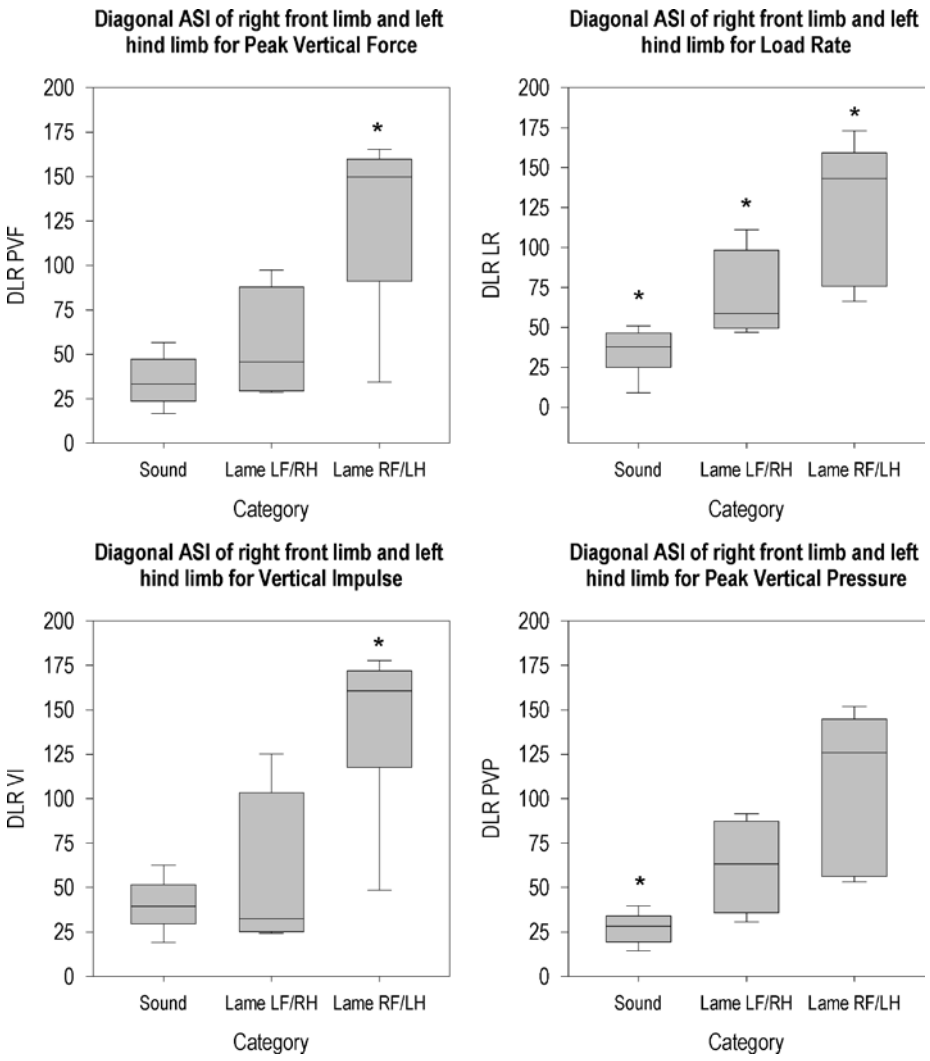

Figure 7. Diagonal ASI for right front limb and left hind limb per group for all parameters. * The ASI of this group differs from the ASIs of the other two groups ($P < 0.05$).

CLT (Figure 8) was higher in lame animals compared to sound animals for all parameters (PVF: t(18) = -6.11, p = 0.000, LR: t(18) = -4.22, p = 0.002), VI: t(18) = -6.80, p = 0.000, PVP: t(18) = -4.60, p = 0.001).

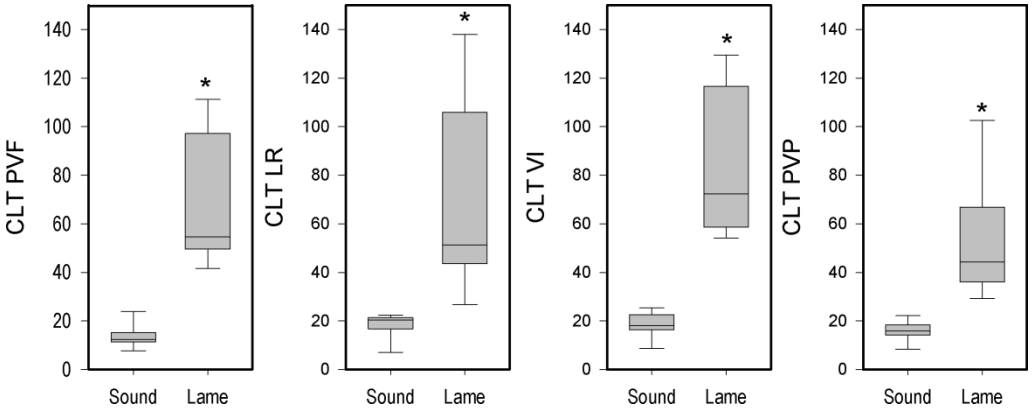

Figure 8. Boxplots for CLT for PVF, LR, VI and PVP for the lame and sound groups. * This group differs from the sound group (P < 0.05).

Correlation with visual scoring

Visual score of the 20 pigs correlated highly with CLT of PVF (r = 0.82, p < 0.05), followed by CLT of VI (r = 0.83, p < 0.05), CLT of LR (r = 0.80, p < 0.05) and CLT of PVP (r = 0.77, p < 0.05).

ROC analysis

The ROC analysis showed distinct cutoff values with 100% sensitivity and specificity for all four parameters. The cutoff value calculated from the ROC analysis was 33.1 for CLT of PVF, 24.0 for CLT of LR, 39.8 for CLT of VI and 25.6 for CLT of PVP.

Discussion

Data collection from both lame and sound pigs appeared relatively simple, as both groups showed exploratory behavior and a strong response to the treats. Lame pigs walked at a slower mean velocity than sound pigs. This is in agreement with findings in sows (Grégoire et al., 2013) and cattle (Chapinal et al., 2009; Flower et al., 2005). Velocity itself may provide an indicator for lameness. However, there is some overlap between lame pigs and sound pigs on this measure. Also, in practice, velocities can only be measured and compared under standardized conditions. It may be difficult to distinguish the different velocities by visual judgment. Lastly, velocity per se does not provide any clue as to

which limb is the lame one. Directional ASIs can provide this information. Velocity, however, may be a first indicator to direct a farmer's or veterinarian's attention to certain pigs; a pig moving exceptionally slow may warrant further examination. Incorporation of "walking speed" as a parameter into lameness scoring protocols therefor may be advisable.

The lame pigs in this study were selected from a commercial breeding farm without prior knowledge of the cause of the lameness. As a result, the group with the lame pigs was rather heterogeneous, with several pathomorphological diagnoses on different locations in the limbs. Since the kinetic parameters we used were representing the forces in the entire limb, we did not expect location of the lesion (proximal or distal in the limb) to influence the magnitude of ASIs. However, the study group was not large enough to make any definitive statements on this subject.

Redistribution of pressure mat parameters

Contralateral

The contralateral asymmetry was assessed from the CFL and CHL parameters. CFL compares the two forelimbs to each other. If a pig is lame on a forelimb, it will reduce the amount of force put on that limb by shifting it towards the other forelimb, and to a lesser extent to the ipsilateral and diagonal hind limbs. Thus, it is possible that in a pig that is lame on a forelimb, the hind limb asymmetry (CHL) also increases due to a mechanical compensation, as there is no pain related lameness in these hind limbs. In our experiment, as expected the CFL was increased in forelimb lame pigs compared to that recorded in sound pigs. The CHL of pigs that were lame on a forelimb was also higher than that of the sound animals. The increased asymmetry in the forelimbs was also found in studies on lame horses (Weishaupt et al., 2006) and dogs (Abdelhadi et al., 2012; Bockstahler et al., 2009) for PVF and VI, and for PVP in lame pigs (Karriker et al., 2013). In both the aforementioned equine and canine studies, the increased asymmetry was mainly due to a significant decrease in PVF and VI in the lame limb. The PVF and VI in the contralateral limb did not increase significantly in lame dogs at walk, but did increase in horses at higher lameness grades.

In agreement with our study, also in forelimb lame dogs and horses the CHL similarly increased for both PVF as well as VI. This may be due to a significant force shift from the lame forelimb to the diagonal hind limb. Moreover, this has been attributed to unloading of the limb ipsilateral to the lame limb (i.e., when RF is lame RH is also unloaded) (Weishaupt, 2008). We also saw a shift towards the diagonal limb in our lame animals, which

would cause asymmetry due to a mechanical lameness also in the hind limbs, even though there is no true pain related lameness present in these limbs.

The load distribution between the two hind limbs is quantified by the CHL. Indeed, the CLH of the hind limb lame pigs in our experiment increased, similar as to earlier findings in hind limb lame dogs, horses and pigs (Fanchon and Grandjean, 2007; Fischer et al., 2013; Karriker et al., 2013; Oosterlinck et al., 2011; Weishaupt et al., 2004). The increased asymmetry may be due to a decreased loading of the lame limb, which was found in horses (Weishaupt et al., 2004) and dogs (Fischer et al., 2013) and additionally due to an increased loading of the contralateral hind limb. The latter mechanism was observed in the study by Fischer et al.(2013), whereas in the study by Weishaupt et al. (2004) it was only observed for VI and not for PVF.

The CFL of animals that were lame on a hind limb was significantly higher than that of sound animals only for VI. This may be because the lameness mainly affects stance time rather that the PVF. This adaptation of timing rather than loading may be a more subtle manifestation of compensation in lame pigs. A significant increase of stance time was found using kinematics in lame sows (Grégoire et al., 2013). In walking dogs an increased CFL in dogs that were lame on a hind limb was found both for PVF and VI (Fischer et al., 2013), but in trotting horses that were lame on a hind limb no increased asymmetry was found (Weishaupt et al., 2004). In the dogs, the ipsilateral forelimb had an increased PVF and the diagonal forelimb had an increased VI, thereby influencing forelimb symmetry.

Ipsilateral

The fore-hind symmetry is assessed by the ILL and ILR. Generally, we expected that front-hind asymmetry would increase on the side of the lesion, as was shown previously in dogs (Bockstahler et al., 2009) and pigs (Karriker et al., 2013). Again, the main reason for this asymmetry was unloading of the lame limb (Abdelhadi et al., 2012; Bockstahler et al., 2009; Fischer et al., 2013; Weishaupt et al., 2006, 2004). With the exception of the study by Fischer (2013), who found an increased PVF in the ipsilateral limb of dogs, no increase in loading of the ipsilateral limb was found in these studies. In our experiment, we also found an increase in asymmetry on the ipsilateral side of the lameness with the exception of ILL of PVP. It may be that the same mechanism that cause lameness in the forelimbs also caused contralateral asymmetry in the hind limbs, namely unloading of the ipsilateral limb (Weishaupt, 2008), causes the difference between the ipsilateral limbs to become smaller, resulting in an increase in asymmetry that is not statistically significant.

There was no increase in ipsilateral symmetry on the side opposite the lesion, except for LR. This is in contrast with the study on walking dogs (Bockstahler et al., 2009), where a significant increase in ipsilateral asymmetry of PVF and VI was seen on the sound side as well as on the lame side. This change only in load rate may represent a subtle manifestation of compensation.

Diagonal

Lameness in the left fore or right hind limb caused an increase in DLL. Lameness in the right fore or left hind limb caused an increase in DLR. This increase was expected, as several studies on horses and dogs have shown redistribution of force away from the lame limb and towards the diagonal limb (Abdelhadi et al., 2012; Bockstahler et al., 2009; Weishaupt et al., 2006). Lameness on a diagonal limb pair, however, did not always influence the other diagonal. The only observable change in ASI of diagonal limbs in pigs that were lame on the limbs outside the diagonal limb pair was seen for LR and PVP. Bockstahler et al. (2009) did not find a similar significant change in diagonal ASIs for the non-lame limbs for PVF and VI, like was found in our study. This may be due to the relatively high degree of lameness of the pigs in our study, which may have caused a larger effect on other limbs compared to the study by Bockstahler et al.

Correlation with visual scoring

Correlations between visual scoring of lameness and CLTs of all parameters were good. This finding is in agreement with the study of Oosterlinck et al. in dogs (2011), who also found good correlations between visual scoring and PVF, VI, and PVP, as recorded using a pressure mat. In that study, PVP was the parameter that correlated lowest with visual gait scores, in contrast to the findings in our study. Oosterlinck et al. (2011)hypothesized that the decrease in limb loading of the lame limb combined with a concurrent decrease in contact area resulted in a “falsely” low pressure value (which is force per unit of area), rendering PVP as a less reliable parameter. A study in horses (Ishihara et al., 2005) that compared visual lameness scoring to force plate, also found significant correlations, particularly with PVF and VI. Quinn et al. (2007), however, did not find correlations between PVF measured by force plate and numerical rating scale scores of three observers scoring lame dogs. Only one out of three observers had a significant correlation between the numerical rating scale score and VI as recorded using a force plate. In that study, the highest correlation was only found when dogs were considerably lame. Our study did not include pigs that had a lameness score of 1 (subtle lameness). This may be a reason for the discrepancy between our findings and those of Quinn et al. (2007). It is also possible that ASIs of kinetic parameters provide better correlation with visual scoring than

absolute values, as a visual evaluation is an interpretation of symmetry of locomotion rather than a measure of absolute limb loading. Often, compensatory load redistribution to the contralateral limb occurs, thus enhancing asymmetrical limb kinetics and kinematics (Katic et al., 2009; Weishaupt et al., 2006, 2004).

ROC analysis

In the present study, we assessed the use of CLT of a set of only four pressure mat parameters to distinguish lame from sound pigs. Lameness has often been described in terms of asymmetry. A previous study by Karriker et al. (2013) found a substantial decrease in symmetry of PVP in an experimental lameness model in sows. However, no cutoff values were estimated yet for these ASIs to distinguish lame pigs from sound pigs. In dogs, however, these cutoff values already have been established. Fanchon and Grandjean (2007)used an instrumented treadmill to compare ASIs for PVF, VI and LR and found PVF to be the only ASI with a high accuracy. Oosterlinck et al. (2011) found ASIs of PVF, VI and contact area to be highly accurate, in contrast to the ASI of PVP. In both studies, it was known that the dogs were lame on the hind limbs. However, in a clinical setting it may not always be clear which is the lame limb. In our study, the pigs were lame only on one of the four limbs. Therefore, we used the CLT as a potential tool to determine the presence of lameness.

A certain degree of asymmetry was present in sound animals as well as in lame animals. No animal was completely symmetrical, which is in agreement with findings in dogs (Colborne et al., 2008) and horses (Oosterlinck et al., 2010a). Using ROC analysis, optimal cut-off points were found for all four overall left-right ASIs, with a 100% sensitivity and specificity. In future studies, it would thus be interesting to assess the performance of the pressure mat as a tool to detect very subtle lameness. In this study, there were no pigs with a lameness score of 1 (very subtle lameness) used, as such pigs are not easily identified on a farm in a practical situation.

In conclusion, lameness in walking pigs causes an increase in contralateral, ipsilateral and diagonal asymmetry, due to unloading of the lame limb combined with redistribution of this load to the non-lame limbs. However, the six ASIs that were studied only took into account two limbs at a time. Animals that were lame on a limb that was not assessed by that ASI did not always have a higher asymmetry than sound animals. In situations where it is unknown which is the lame limb, a method that takes into account all four limbs is needed. An example of such a method is the use of overall left-right ASIs.

Using the overall left-right ASIs we were able to distinguish lame pigs from sound pigs with 100% sensitivity and specificity, which correlated well with subjective lameness scores. They may provide a future objective method to assess lameness and the effect of interventions on the presence of lameness.

Methods

The study was reviewed and approved by the local ethical committee of Utrecht University (DEC no 2012.III.05.04), The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

Animals

A group of n = 10 clinically lame and of n = 10 sound Topigs 20 × Tempo pigs (6 male, 4 female per group) were selected by a veterinarian at a commercial breeding farm. Only pigs that were clinically lame on one limb, that could stand and walk unaided and that did not show signs of any other disease were selected. The group of sound pigs was assessed as one total batch, while due to logistics the lame pigs were evaluated in 3 batches 2 weeks apart. All pigs were clinically examined at the farm by a veterinarian to make sure they fitted the inclusion criteria for this study, before they were transported to the research facility of the Veterinary Faculty, Department of Farm Animal Health, Utrecht University.

Housing

The pigs were housed at the research facility of Utrecht University. They were kept in small subgroups of 3-5 pigs in pens with closed concrete floors measuring 153 cm × 256 cm that were covered with sawdust. The pigs were provided with 11 hours of light per day (from 7 a.m. to 6 p.m.) from both daylight and artificial lighting. They were housed in a stall with an ambient temperature between 22 and 24°C and one extra heatlamp per pen was provided. The animals had ad libitum access to food (Groeiporco, De Heus Animal Nutrition, Ede, The Netherlands) and water, and were provided with enrichment toys (plastic ball, metal chain) during the entire experiment.

Data recording

Upon arrival at the research facility, the lame pigs (first batch n = 4 pigs, second batch n = 3 pigs, third batch n = 3 pigs) were put together in one single pen, without any sound

animals in the same pen. The sound pigs were grouped according to size in two subgroups of 5 pigs. All pigs were allowed to acclimatize for one day before entering the experiment. At the starting day of the experiment, piglets were weighed and clinically examined (breathing rate, heart rate, rectal temperature, assessment of skin, mucous membranes and lymph nodes).

The pressure mat recordings were performed using a Footscan® 3D Gait Scientific 2 m system (RSscan International, Olen, Belgium) with an active sensor surface of 1.95 m × 0.32 m containing 16384 sensors (2.6 sensors per cm^2), with a sensitivity of 0.27-127 n/cm^2 and a measuring frequency of 126 Hz. The pressure mat was connected to a laptop with dedicated software (Footscan Scientific Gait 7 gait 2^{nd} generation, RSscan International, Olen, Belgium). The mat was placed in a custom-built runway as used by Meijer et al. (2014a). The pressure mat was calibrated according to the manufacturer's instructions by a person weighing approximately 70 kg.

Visual scoring was performed according to the method described by Main et al. (2000b). This scoring system yields a score from 0 to 5, with 0 being a sound individual and 5 an extremely lame pig. Observations were first made in the home pen of the piglets without disturbing them. Posture, behavior and gait were marked. After that, the observer approached the pigs and opened the pen to note the behavioral response to this stimulus. Finally, if pigs had not risen yet, the observer encouraged the pig to stand up so that locomotion could be scored.

For the pressure mat analysis, one pig at a time was let out of its pen and was allowed to walk freely to the holding area. Very lame pigs that were reluctant to leave the pen were carried. Once inside the holding area, the pig was allowed to acclimatize for one minute. After this, the door to the runway was opened and the pig could walk to the holding area at the other side. All pigs eventually started to explore their surroundings and crossed the runway. Every time they did, they were rewarded with candy. Exploratory behavior together with rewards was sufficient to collect three correct runs per pig. A run was considered correct when the pig walked (gait confirmed by duty factor) across the runway without stopping at a steady velocity and looking straight ahead.

After all data had been collected, the pigs were euthanized. They were sedated using a 2 mg/kg intramuscular injection of Azaperone (Stresnil, Elanco Animal Health, Greenfield, USA) and subsequently euthanized by intracardial injection of 200 mg/kg Pentobarbital (Euthanimal, Alfasan, Woerden, The Netherlands). Gross pathology was performed at the Department of Pathobiology of the Faculty of Veterinary Medicine of Utrecht University, with specific attention paid to their limbs, and in particular the lame limb.

Data analysis

The collected footprints from their claws in the three runs were manually assigned to left fore (LF), right fore (RF), left hind (LH) and right hind (RH) limb using the software provided by the pressure plate manufacturer. PVF (N), LR (N/s), VI (Ns) and PVP (N/cm²) were normalized for body mass.

ASIs were calculated for each variable using modifications of the formula introduced by Oomen et al. (2012) (Figure 9).

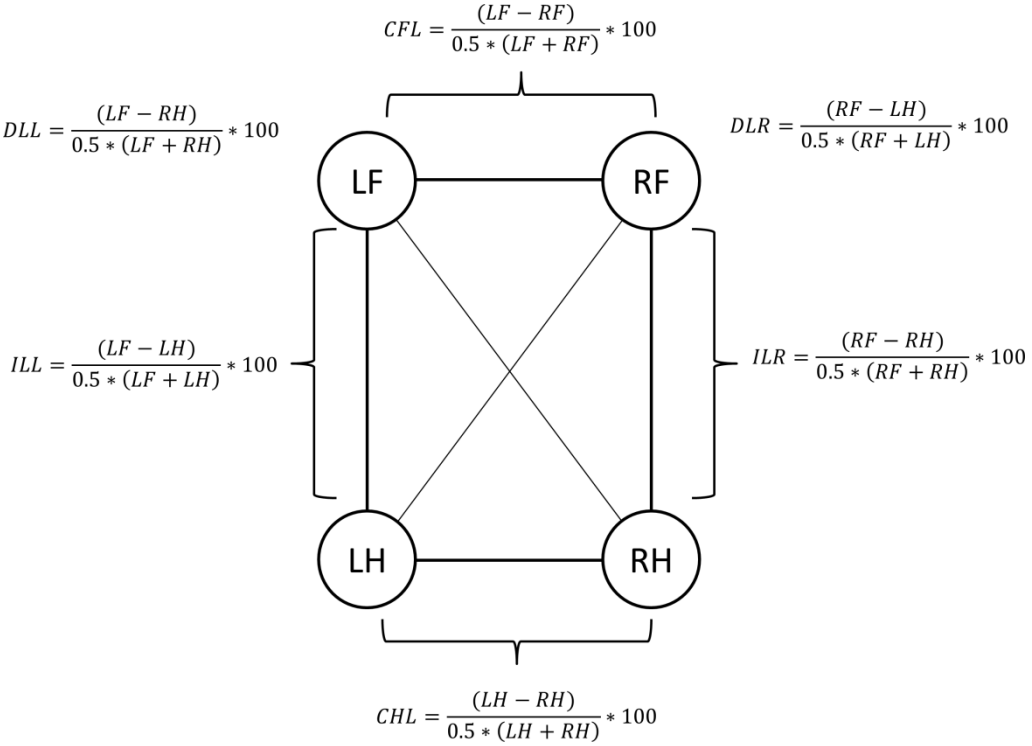

Figure 9. Formulas used for calculation of the various ASIs. CFL: contralateral forelimbs, CHL = contralateral hind limbs, ILL = ipsilateral left limbs, ILR = ipsilateral right limbs, DLL = diagonal left fore and right hind limbs, DLR = diagonal right fore and left hind limbs. LF = left fore, RF = right fore, LH = left hind, RH = right hind.

The total left-right ASI (CLT) compared the left limbs to the right limbs and was calculated using the formula:

$$CLT = \frac{(LF+LH)-(RF+RH)}{0.5*((LF+LH)+(RF+RH))} * 100$$

These formula's for ASIs yield a score between -200 and + 200. Both extreme values indicate very severe (non-weight bearing) lameness. The direction of the extreme (negative or positive) indicated the direction of the weight redistribution. An ASI of 0 indicates perfect symmetry.

To determine redistribution, the ability of ASIs to identify lame pigs, and the correlation between visual scoring and ASIs, the absolute value of the ASIs was used, removing the distinction between right- or left-sided asymmetry This yields a score between 0 and 200, with higher values indicating relatively higher loading of the left or right limb and 0 indicating perfect symmetry. Mean ASIs were calculated from the 3 ASIs per pig.

Statistics

The body mass, velocity and CLT of the lame and sound groups were compared using an independent-samples *t*-test. The data for body mass and velocity had equal variances in both groups according to Levene's test, but the data from the CLT did not, therefore the non-parametric Mann-Whitney U test was used.

To assess whether redistribution was taking place, the animals were divided into 3 groups for the six ASIs comparing two limbs (CFL, CHL, ILL, ILR, DLL, DLR): sound animals, animals that were lame on a limb that was assessed by that particular ASI and animals that were lame on a limb that was not assessed by that ASI. For example, for the CFL there were three groups: sound animals, animals that were lame on the left front limb or the right front limb (the limbs that are assessed by CFL) and animals that were lame on the left hind limb or the right hind limb (the limbs that are not assessed by the CFL). Since the data were not distributed normally (confirmed by Kolmogorov-Smirnov test), a Kruskall-Wallis test was used to compare the three groups. A Mann-Whitney test with Bonferroni correction was performed as a post-hoc test to assess the differences within groups.

Spearman's rank correlation coefficient was used to evaluate correlations between visual scores and CLT's.

Receiver-operated curve analysis (Shapiro, 1999) was performed to assess the performance of each of the CLT's as diagnostic test in the diagnosis of lameness. The sensitivity (y axis) was plotted against 1-specificity (x-axis) for each possible cutoff value. A diagonal

line where sensitivity is equal to 1- specificity represents a discriminating ability of the test that is no better than chance. The top left corner represents 100% sensitivity and specificity. The resulting area under the curve (AUC) was used to assess the performance of each of the CLT's.

All data are presented as means ± SD. Statistical significance was set at $p < 0.05$.

Abbreviations

LF, Left forelimb; RF, Right forelimb; LH, Left hind limb; RH, Right hind limb; PVF, Peak Vertical Force; LR, Load Rate; VI, Vertical Impulse; PVP, Peak Vertical Pressure; ASI, Asymmetry index; CLT, Asymmetry of both left limbs (LF and LH) vs. both right limbs (RF and RH); CFL, Asymmetry of contralateral forelimbs; CHL, Asymmetry of contralateral hind limbs; ILL, Asymmetry of left ipsilateral limbs; ILR, Asymmetry of right ipsilateral limbs; DLL, Asymmetry of diagonal fore- and hindlimb (LF vs. RH); DLR, Asymmetry of diagonal fore- and hindlimb (RF vs. LH)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

EM contributed to study design and data collection, analysed the data and drafted the manuscript. MO provided advice on pressure mat data collection and analysis, and critically revised the manuscript. AvN critically revised the manuscript. WB contributed to study design, provided advice on automated gait analysis and critically revised the manuscript. FJS aided in writing the first draft of the manuscript, provided advice on data analysis and critically revised the manuscript. All authors read and approved the final manuscript

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Additional files. Full test statistics and exact p-values for all ASI's

Additional file 1: Contralateral ASI's

variabele	KW chi-sq	P-value	group	Mann-whitney U	p-value
CFL PVF	11.61	0.003	Sound-lame front	40	0.006
			Sound- lame hind	48	0.058
			Lame front- lame hind	24	0.014
CFL LR	11.09	0.004	Sound-lame front	40	0.006
			Sound- lame hind	46	0.093
			Lame front- lame hind	24	0.014
CFL VI	12.19	0.002	Sound-lame front	40	0.006
			Sound- lame hind	50	0.034
			Lame front- lame hind	24	0.014
CFL PVP	7.01	0.030	Sound-lame front	38	0.013
			Sound- lame hind	37	0.481
			Lame front- lame hind	21	0.070
CHL PVF	15.48	0.000	Sound-lame hind	60	0.001
			Sound-lame front	40	0.006
			Lame front- lame hind	2	0.043
CHL LR	14.58	0.001	Sound-lame hind	60	0.001

			Sound-lame front	40	0.006
			Lame front- lame hind	7	0.337
CHL VI	15.48	0.000	Sound-lame hind	60	0.001
			Sound-lame front	40	0.006
			Lame front- lame hind	2	0.043
CHL PVP	14.29	0.001	Sound-lame hind	60	0.001
			Sound-lame front	40	0.006
			Lame front- lame hind	12	1.000

Additional file 2: Ipsilateral ASI's

variabele	KW chi-sq	P-value	group	Mann-whitney U	p-value
ILL PVF	11.09	0.004	Sound-lame left	40	0.006
			Sound-lame right	46	0.093
			Lame left-lame right	24	0.014
ILL LR	9.07	0.011	Sound-lame left	40	0.006
			Sound-lame right	44	0.143
			Lame left-lame right	20	0.110
ILL VI	8.74	0.013	Sound-lame left	38	0.013
			Sound-lame right	46	0.093
			Lame left-lame right	21	0.070
ILL PVP	3.86	0.145	Sound-lame left	32	0.104

			Sound-lame right	39	0.357
			Lame left-lame right	19	0.166
ILR PVF	6.32	0.042	Sound-lame right	51	0.026
			Sound-lame left	16	0.621
			Lame left-lame right	3	0.070
ILR LR	8.22	0.016	Sound-lame right	52	0.020
			Sound-lame left	35	0.040
			Lame left-lame right	6	0.241
ILR VI	7.84	0.020	Sound-lame right	53	0.015
			Sound-lame left	14	0.437
			Lame left-lame right	2	0.043
ILR PVP	9.53	0.009	Sound-lame right	56.5	0.005
			Sound-lame left	32	0.104
			Lame left-lame right	6	0.241

Additional file 3: Diagonal ASI's

variabele	KW chi-sq	P-value	group	Mann-whitney U	p-value
DL PVF	9.52	0.009	Sound-Lame left front or right hind	40	0.006
			Sound- Lame right front or left hind	37	0.481
			Lame left front or right hind- lame right front or left hind	24	0.014

DLL LR	10.94	0.004	Sound-Lame left front or right hind	40	0.006
			Sound- Lame right front or left hind	51	0.026
			Lame left front or right hind- lame right front or left hind	19	0.166
DLL VI	9.15	0.010	Sound-Lame left front or right hind	40	0.006
			Sound- Lame right front or left hind	29	0.957
			Lame left front or right hind- lame right front or left hind	24	0.014
DLL PVP	13.90	0.001	Sound-Lame left front or right hind	40	0.006
			Sound- Lame right front or left hind	55	0.008
			Lame left front or right hind- lame right front or left hind	24	0.014
DLR PVF	9.14	0.010	Sound- Lame right front or left hind	55	0.008
			Sound-Lame left front or right hind	29	0.229
			Lame left front or right hind- lame right front or left hind	2	0.043
DLR LR	14.66	0.001	Sound- Lame right front or left hind	60	0.001
			Sound-Lame left front or right hind	38	0.013

			Lame left front or right hind- lame right front or left hind	2	0.043
DLR VI	9.33	0.009	Sound- Lame right front or left hind	56	0.006
			Sound-Lame left front or right hind	19	0.944
			Lame left front or right hind- lame right front or left hind	1	0.025
DLR PVP	13.01	0.001	Sound- Lame right front or left hind	60	0.001
			Sound-Lame left front or right hind	35	0.040
			Lame left front or right hind- lame right front or left hind	4	0.110

4

The clinical effects of buprenorphine on open field behaviour and gait symmetry in healthy and lame weaned piglets

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Abstract

Lameness in pigs decreases animal welfare and economic profit for the farmer. An important reason for impaired welfare in lame animals is pain due to lameness-causing processes. Until now, no direct measurement of pain is possible in animals, and methods to indirectly detect and quantify the amount of pain an animal is experiencing thus are urgently needed.

Therefore, in this study, two methods to assess pain associated with lameness in pigs were evaluated to determine if they were sensitive enough to detect a lameness reduction as an effect of an experimental analgesic medication. For this, asymmetry associated with lameness was objectively quantified using pressure mat kinetic parameters [Peak Vertical Force (PVF), Load Rate (LR), Vertical Impulse (VI) and Peak Vertical Pressure (PVP)], whereas locomotor activity was assessed in an open field test. A dose of 0.04 mg/kg buprenorphine, a strong analgesic, was used to treat 10 lame pigs, while 8 other lame pigs, treated with physiological saline solution served as controls.

Buprenorphine decreased lameness-associated asymmetry for pressure mat LR ($t(9)=4.179$, $P=0.002$), VI ($t(9)=4.122$, $P=0.003$) and PVP ($t(9)=4.925$, $P=0.001$) and increased activity of the lame pigs in the open field ($t(9)=-2.746$, $P=0.023$), while saline-treated animals did not show any changes in asymmetry and became less active in the open field ($t(7)=10.963$, $P=0.000$).We therefore concluded that measurement of gait asymmetry by pressure mat analysis and locomotor activity in an open field test both are sensitive enough to detect the analgesic effects of buprenorphine when used to treat moderate to severe clinical pain in a relative small group of affected pigs. The methods used in this study may also provide promising additional tools for future research into early pain recognition and lameness treatment in pigs.

Keywords: Kinetic; Pressure mat; Lameness; Behaviour; Pigs.

Introduction

Lameness is a common problem in swine husbandry and results in both a reduced animal welfare and economic losses to the farmer (S. S. Anil et al., 2009; T. B. Jensen et al., 2012; KilBride et al., 2009). The decreased animal welfare is due to pain, as well as a decreased ability to show normal behaviour (S. Anil et al., 2009). It is important to detect and adequately treat these lamenesses, which are usually caused by painful processes. Lame pigs may also provide an interesting model for the testing of potentially analgesic treatments for use in humans.

To assess pain associated with lameness in pigs, objective and sensitive methods are required. Several methods to assess pain have been developed, mostly in rodents. Many of these methods focus on reflex testing of nociception (Di Giminiani et al., 2013; Mohling et al., 2014; Tapper et al., 2013). A drawback of testing nociception in this way, however, is that it does not necessarily reflect the perception of pain by the animal (Cobos and Portillo-Salido, 2013; Mao, 2012). Therefore, there is a need for additional methods to indirectly quantify pain experience (Cobianchi et al., 2014; Cobos and Portillo-Salido, 2013). In this study, we used two of such methods.

First, we assessed locomotion asymmetry using kinetic parameters collected with a pressure mat. Pressure mat analysis of kinetic parameters is a useful tool to detect lameness in several species (Karriker et al., 2013; Lequang et al., 2010; Maertens et al., 2011; Oosterlinck et al., 2011; Oosterlinck et al., 2010a, 2010b). It is especially useful for pigs since it is possible to collect data on several footfalls in one run, and thus to calculate asymmetry indices (ASI's) from one run. This minimises the effects of velocity on kinetics, which are difficult to control in these animals (Meijer et al., 2014a, 2014b) .

Second, we quantified spontaneous locomotor activity in an open field test. Open field activity has been used extensively in rodents to measure spontaneous exploratory behaviour. It has also been used in pigs, for example to study the effect of head trauma, early isolation and various substances on exploratory activity (Fraser, 1974; Friess et al., 2007; Kanitz et al., 2004; Thodberg et al., 1999; Van Der Staay et al., 2009a).

To help validate pressure mat analysis and the open field test for assessment of pain associated with lameness we compared measurements from 18 clinically lame pigs treated with 0.04 mg/kg buprenorphine (a potent analgesic acting as a mu-opioid antagonist) and lame pigs treated with a placebo (physiological saline). Buprenorphine has been shown to be effective in pigs (Hermansen et al., 1986; Rodriguez et al., 2001) and evidence for unwanted behavioural side-effects is limited to small increases in motor behaviour in response to a dose of 0.10 mg/kg IM buprenorphine (Hermansen et al., 1986). However, no changes in behaviour were reported by Harvey-Clark et al. (2000)

when they compared 0.10 mg/kg IM buprenorphine with 25 and 50 µg/h transdermal fentanyl patches.

We hypothesised that since pain is the most obvious reason for an animal to display lameness, the administration of an analgesic should at least partially restore symmetric gait, measured by the pressure mat. We also expected that lame pigs would show increased activity in the open field test after treatment with buprenorphine. Animals that experience pain tend to be less active (Weary et al., 2006) and lame sows spend more time laying down (Grégoire et al., 2013). The animal tries to avoid pain by putting less weight on the affected limb and is less motivated to walk around. Changes in locomotor activity in the open field test may therefore reflect changes in the amount of pain an animal experiences.

In a pilot study (see "Supplemental materials") we did not find any significant influences of a dose 0.04 mg/kg buprenorphine IM on any of the outcome parameters used in the present study, therefore we chose this dose for use in the current study.

Material and Methods

The study was reviewed and approved by the local ethical committee of Utrecht University (no. 2012.III.05.04 , date of approval 23 May 2012), and was conducted in accordance with the recommendations of EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

Animals

Eighteen lame Topigs 20 x Tempo pigs were selected from a commercial farm by a veterinarian. Due to logistic reasons, the pigs were collected in five batches, each two weeks apart. Inclusion criteria were: 3- to 10-week-old pigs, which were clinically lame in one limb, but were able to stand and walk unaided, were without any concurrent disease and were not treated with antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs) for at least 48 hours prior to selection.

Housing

The pigs were housed at the research facility of Utrecht University in groups of 3 or 4 pigs in pens with sawdust-covered solid concrete floors measuring 153 cm x 256 cm. They were provided with 11 hours of light per day (7 AM to 6 PM) from both daylight and artificial lighting. Ambient temperature ranged from 22 to 24°C. All pigs had *ad libitum* access to food (Groeiporco, De Heus Animal Nutrition BV), water and toys (plastic ball, metal chain).

Treatment

The analgesic used was Buprecare Multidosis (AST Farma BV), containing 0.3 mg buprenorphine hydrochloride per ml. Physiological saline solution (9 g sodium chloride per litre) was used as control compound (Eurovet Animal Health BV). A researcher not involved in data collection assigned each animal randomly to either the treatment or control groups and prepared the syringes with either buprenorphine or saline solution in the same volumes. The two solutions could not be distinguished by the person administering them to the pigs.

Pigs were allowed to acclimatise for 24 hours. At the start of the experiment, the complete set of measurements (clinical exam, weighing, visuals scoring of gait, open field testing and pressure mat measurements) was performed. The next day, twenty-four hours later, the pigs received an intramuscular injection with either 0.04 mg/kg buprenorphine or an equal volume of saline solution.1.5 hours after injection with buprenorphine or control solution, the set of measurements was repeated. This meant that pre-and post-treatment measurements were taken on comparable times of the day.

Data collection

First, data that could be collected without disturbing pigs were noted (breathing rate and skin colour). Then, visual scoring of gait according to the protocol by Mail et al. (2000b) was performed. Heart rate, rectal temperature, mucous membranes, lymph nodes (to assess if the animals were clinically healthy) were examined and bodymass was measured. All measurements were performed by an experienced veterinarian.

Open field testing was performed in a pen (153 x 256 cm bordered by a 90 cm high wall) in a separate room. A video camera was mounted approximately 2.5 meters above the pen. The pig was transported to the pen using a cart, and was placed in the open field where testing immediately began. After 5 minutes the recording was stopped and the pig was removed from the pen. The pen was rinsed with clean tap water between each pig.

The pressure mat had an active sensor surface of 1.95 m × 0.32 m containing 16384 sensors with a pressure range of 0.27-127 n/cm^2 and a sampling frequency of 126 Hz (RSscan International NV) and was used in the same setup that was previously described by Meijer et al (2014a).

Pigs were guided one by one to the holding area. After one minute, the door leading to the runway was opened. Exploratory behaviour combined with candy rewards encouraged the pigs to walk across the runway. Test runs were repeated for each pig until three runs met the following selection criteria: the pig walked along the runway at a consistent

velocity, looking straight ahead and without stopping. Walking was confirmed from a duty factor (the percentage of the total gait cycle the foot has contact with the ground) greater than 0.5.

After data collection, the pigs were sedated with 2 mg/kg intramuscular Azaperone (Stresnil, Elanco Animal Health) and subsequently euthanized by intracardial injection of 200 mg/kg Pentobarbital (Euthanimal, Alfasan Diergeneesmiddelen BV). Gross pathology was performed at the Department of Pathobiology of the Faculty of Veterinary Medicine of Utrecht University, with specific attention being paid to the affected limb.

Data analysis

Open field behaviour: Videos were scored by an observer using the purpose-built scoring program OBSERVE (Van Der Staay et al., 2009b). The screen was divided into 12 equal-sized squares and each time the pig crossed a line, this was scored. If any notable behaviour occurred (for example rearing or jumping against pen wall), this was also registered. Total amount of line crossings in 5 minutes was used as an index for locomotor activity.

Pressure mat: For the pressure mat data, claw strikes from the 3 valid runs were manually assigned to left fore (LF), right fore (RF), left hind (LH) and right hind (RH) limb. Four kinetic parameters were collected: Peak Vertical Force (PVF, N), Load Rate (LR, N/s), Vertical Impulse (VI, Ns) and Peak Vertical Pressure (PVP, N/cm^2). The total left-right asymmetry index (ASI) was calculated for each of these parameters using a formula modified from Oomen et al (2012):

$$ASI = \frac{(LF + LH) - (RF + RH)}{0.5 * \big((LF + LH) + (RF + RH)\big)} * 100$$

This formula yields a dimensionless ratio between left and right pressure mat parameters. An ASI of 0 indicates perfect symmetry; the extreme values of -200 or +200 indicate non-weight-bearing lameness on either the left or right side. Absolute ASI values were used for subsequent analysis. To assess the effect of treatment on the parameters, the differences between pre-and post-treatment (difASI for asymmetry indices and difOF for open field activity) were calculated.

Statistical analysis

Normality of the data was assessed using Q-Q plots and Kolmogorov-Smirnov test.

We assessed if pre-and post-treatment levels of ASIs and open field activity differed in the two treatment groups using either a paired-samples t-test for normally distributed data or Wilcoxon's signed rank test for non-normally distributed data. We then checked if

treatment influenced difOF and the difASI of kinetic parameters. An independent-samples *t*-test was used for normally distributed data and a Mann-Whitney U test for non-normally distributed data.

All data were analysed using SPSS version 20.0 for Windows (IBM) and R version 3.1.0 (R Foundation for Statistical Computing) and are presented as means ± SD. Statistical significance was set at $P < 0.05$.

Results

The pigs weighed 10.9 ± 5.3 kg (mean ± SD). General clinical examination did not show any abnormalities in any of these animals, except that all of these pigs were lame with a visual score ranging from 1 (abnormal stride length, movements no longer fluent) to 4 (may not place affected limb on floor). The post-mortem pathological findings of all the pigs are presented in Table 1; no pathology was found in unaffected limbs.

For the measured parameters, no significant differences were found between the means for treatment and placebo groups before treatment (Table 2) (PVF: $t(16)= 0.324$, $P=0.750$, LR: $U=25$, $P=0.183$, VI: $t(16)= 0.094$, $P=0.926$, PVP: $t(16)=0.098$, $P=0.923$, open field crossings: $t(16)=-1.928$, $P=0.072$)). LR and OFT show differences (n.s.) with high variance. Pre-and post-treatment values are depicted in Figures 1-5.

Table 1. Lame limb, diagnosis at necropsy and localization of lameness in lame pigs

ID	Lame limb	Diagnosis at necropsy	Location within limb	Lameness score
25	RF	Periarthritis	Carpal joint	2
26	RH	Periarthritis	Tarsal joint	3
27	RH	Periartritis + Arthritis	Tarsal joint	4
28	LF	Ankylosis/Fracture	Elbow joint	2
29	LF	Periartritis	Distal interphalangeal joint	4
30	RH	Arthritis	Metatarsophalangeal joint	3
31	LH	Arthritis	Metatarsophalangeal joint	3
32	RH	Arthritis	Tarsal joint	2
33	RF	Arthritis	Elbow joint	1
34	RH	Periarthritis	Distal interphalangeal joint	2
35	LH	Periarthritis	Tarsal + Knee joint	2
36	LF	Artritis + periartritis	Carpal joint	1
37	RF	Osteomyelitis, arthritis, peri-artritis	Shoulder joint + humerus	2
38	RF	Periarthritis + arthritis	Metacarpophalangeal joint	3
39	RF	Periartritis	Elbow joint	4
40	RH	Periartritis + artritis	Tarsal joint	3
41	LH	Arthritis	Metatarsophalangeal joint	4
42	LF	Periartritis	Carpal joint	3

Table 2. There were no statistical differences between the two treatment groups before the start of experiment.

Parameter	Treatment	Mean ± SD	Test Statistic	*P* value
Peak Vertical Force	Control	57.10 ± 31.60	$t(16)= 0.324$	0.750
	Buprenorphine	61.72 ± 28.72		
Load Rate	Control	46.43 ± 32.33	$U=25$	0.183
	Buprenorphine	71.77± 37.46		
Vertical Impulse	Control	74.01 ± 32.20	$t(16)= 0.094$	0.926
	Buprenorphine	75.40 ± 30.74		
Peak Vertical Pressure	Control	44.73 ± 30.93	$t(16)=0.098$	0.923
	Buprenorphine	45.95 ± 21.45		
Open Field Crossings	Control	117.38 ± 37.22	$t(16)=-1.928$	0.072
	Buprenorphine	76.70 ± 49.37		

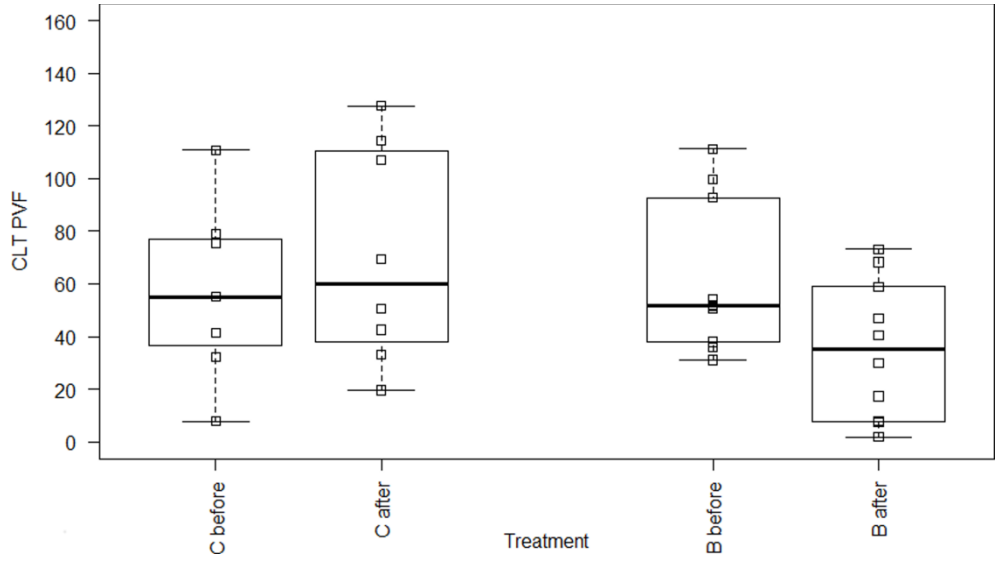

Figure 1. Boxplots for ASI of Peak Vertical Force before and after treatment with either control (n=8) or buprenorphine (n=10).

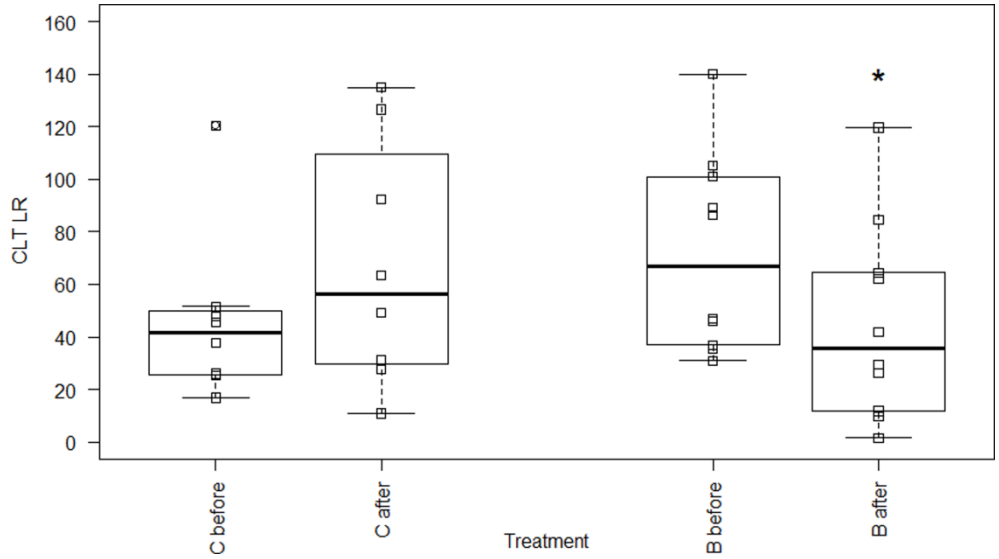

Figure 2. Boxplots for ASI of Load Rate before and after treatment with either control (n=8) or buprenorphine (n=10). Animals treated with buprenorphine became less asymmetric. *significantly lower than buprenorphine group before treatment.

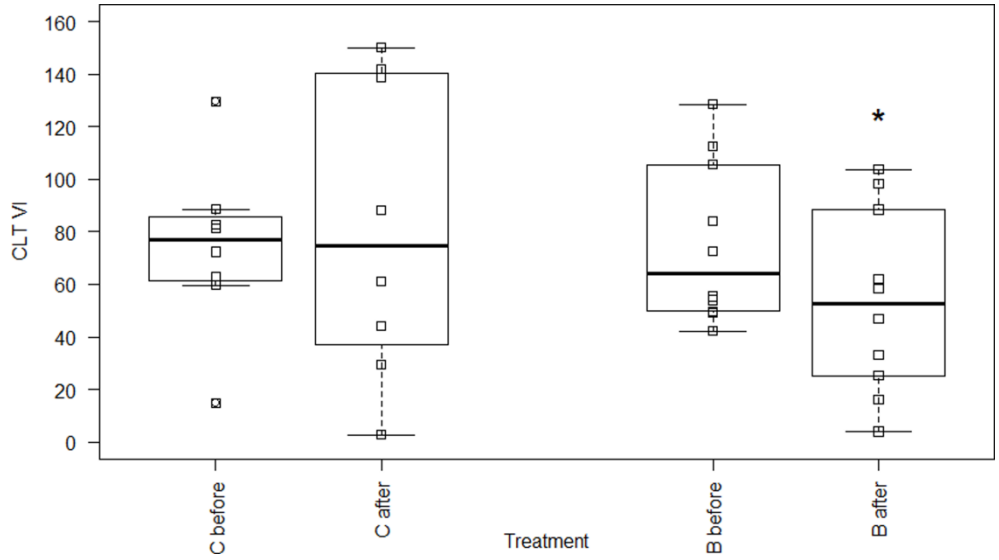

Figure 3. Boxplots for ASI of Vertical Impulse before and after treatment with either control (n=8) or buprenorphine (n=10). Animals treated with buprenorphine became less asymmetric. *significantly lower than buprenorphine group before treatment.

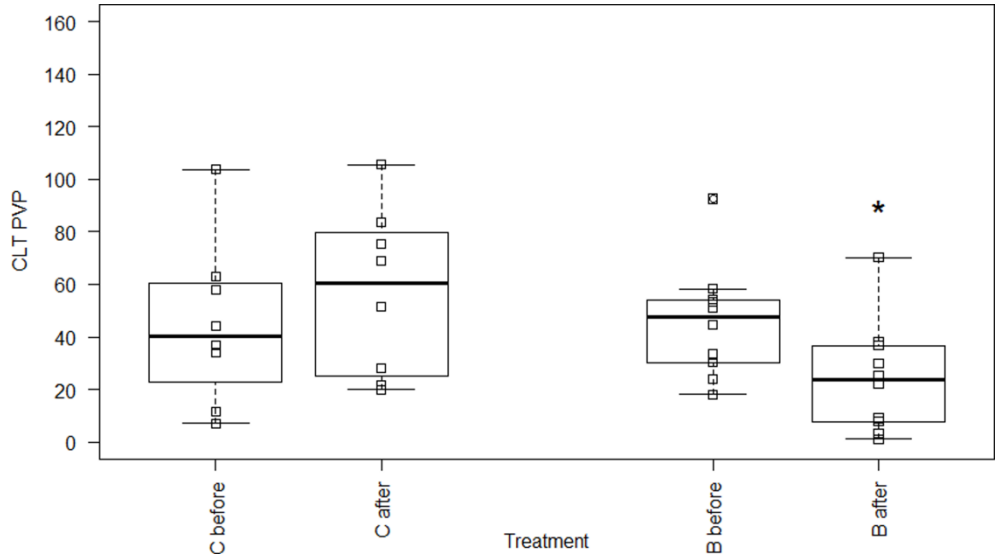

Figure 4. Boxplots for ASI of Peak Vertical Pressure before and after treatment with either control (n=8) or buprenorphine (n=10). Animals treated with buprenorphine became less asymmetric. * significantly lower than buprenorphine group before treatment.

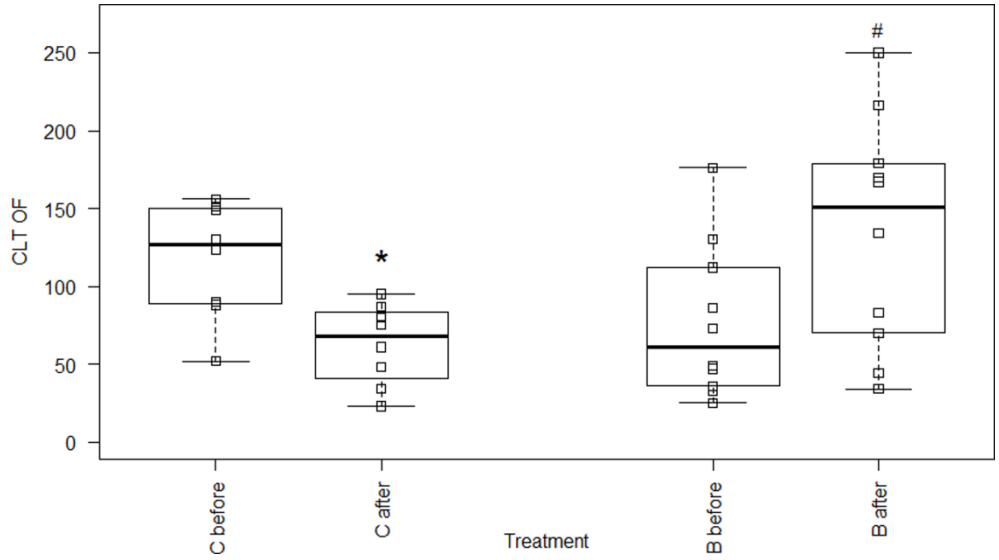

Figure 5. Boxplots for open field crossings before and after treatment with either control (n=8) or buprenorphine (n=10). Animals treated with the control became less active, animals treated with buprenorphine became more active. *significantly lower than control group before treatment, # significantly higher than buprenorphine group before treatment.

The saline-treated group did not show any difference between pre-and post-treatment ASIs (PVF t(7)=-0.490, *P*=.639, LR t(7)=-1.189, *P*=0.273, VI t(7)=-0.489, *P*=0.640, PVP t(7)= -1.036, *P*=0.335), but did show a decrease in open field activity (t(7)=10.963, *P*=0.000). After treatment, all ASIs of kinetic parameters were lower in the buprenorphine-treated group except for PVF (PVF Z=-1.784, *P*=0.074), LR t(9)=4.179, *P*=0.002, VI t(9)=4.122, *P*=0.003, PVP t(9)=4.925, *P*=0.001). The buprenorphine-treated group was also more active in the open field (t(9)=-2.746, *P*=0.023).

Treatment with buprenorphine affected gait on the pressure mat [DifASI, PVF (U=16, *P*=0.033), LR (U=14, *P*=0.021) and PVP (U=15, *P*=0.026] and locomotor behaviour in the open field [DifOF (U=2, *P*=0.001)]. The difference between pre-and post-treatment ASIs was larger in the buprenorphine-treated group than in the control group. In the open field, the difference between pre- and post-treatment was positive for the control group (they became less active) and negative for the treatment group (they became more active). DifASI of Vertical Impulse was not affected by buprenorphine (t(16)=1.72, *P*=0.122). (Table 3)

Table 3. The effects of treatments on the difference between pre-and post-treatment values for asymmetry indices (difASI) and open field crossings (dif Open Field Crossings). A positive number for difASI means improvement (the pigs became less asymmetric). A positive number for dif Open Field Crossings means less activity.

Parameter	Treatment	Mean ± SD	Test Statistic	*P* value
difASI Peak Vertical Force	Control	-13.46 ± 38.99	*U*=16	0.033
	Buprenorphine	26.54 ± 11.67		
difASI Load Rate	Control	-20.62 ± 49.04	*U*=14	0.021
	Buprenorphine	26.60 ± 20.13		
difASI Vertical Impulse	Control	-8.07 ± 46.69	*t*(16)=1.72	0.122
	Buprenorphine	21.76 ± 16.69		
difASI Peak Vertical Pressure	Control	-12.13 ± 33.12	*U*=15	0.026
	Buprenorphine	21.56 ± 13.79		
dif Open Field Crossings	Control	9.75 ± 37.21	*U*=2	0.001
	Buprenorphine	-22.20 ± 80.18		

Discussion

Using both open field test and pressure mat analysis we were able to detect the effect of 0.04 mg/kg intramuscular buprenorphine on lame pigs when compared with placebo treatment. The onset of action of this i.m. dose was within 1.5 hrs and the duration of its effective action exceeded 3 hrs after the start of the treatment. The onset and duration of the pharmacological effects of buprenorphine are within the ranges found in previous studies (onset 30-60 min, duration 6-24 hrs) (Harvey-Clark et al., 2000; Hermansen et al., 1986). The buprenorphine-treated lame pigs showed increased activity in the open field compared to activity levels before administration of analgesia and compared to lame pigs that were treated with saline. Animals that experience pain tend to be less active (Weary et al., 2006) and lame sows spend more time laying down (Grégoire et al., 2013). The animal tries to avoid pain by putting less weight on the affected limb and is less motivated to walk around. Analgesia was expected to antagonise the pain-induced reduction in activity, and the open field test was able to detect this effect. Lame pigs treated with buprenorphine showed increased activity in the open field compared to that before treatment and compared to the lame control pigs that were treated with saline. Interestingly, the control-treated pigs showed a decrease in open field activity after treatment. The decreased activity with repeated testing has been shown in pigs before (Donald et al., 2011).

Pressure mat analysis in lame animals has shown that lameness results in an increased asymmetry of gait (Fanchon and Grandjean, 2007; Karriker et al., 2013; Lequang et al., 2010; Maertens et al., 2011; Meijer et al., 2014b; Oosterlinck et al., 2011). This asymmetry is partly caused by unloading of the lame limb by the animal in an attempt to reduce pain resulting from weight being put on the affected limb. Although the administration of buprenorphine reduced the amount of asymmetry in the lame animals in this study, it did not return ASIs to values previously found in sound pigs (Meijer et al., 2014b). Even though pain is an important component in causing lameness, it may not be the only reason for asymmetric gait in animals. Mechanical restrictions (for example in deforming arthritis) and neurogenic damage may also result in an asymmetric gait and thus lameness. Therefore, the current method of measuring asymmetry cannot directly be translated to the amount of pain experienced by an animal. Another reason why ASIs did not return to normal values may be due to incomplete analgesic effects of buprenorphine on the conditions causing the lameness in these pigs. Most animals suffered from inflammatory conditions, in which buprenorphine may not be very effective (Rodriguez et al., 2001).

The lack of a treatment effect of buprenorphine between pre- and post-treatment PVF and on difASI VI is most likely due to the small, heterogeneous group of animals that was

used in this study. Visual lameness scores already showed that some animals were much more lame than others, ranging from score 1 to 4. The location of the lesion within the limb as well as the staging of the lesion (acute vs chronic) varied between animals (as confirmed post-mortem). Although subjects were randomly assigned to treatment group, and no pre-treatment differences existed for the studied parameters, it is possible that the variability within the subjects may have masked the treatment effect for pre- versus post-treatment for PVF and difASI VI. Based on post-hoc power calculations, in order to also show a treatment effect for these two variables with a power of 0.8, 26 and 22 animals should be included in the treatment group, respectively. In order to reduce the amount of animals needed, a more homogenous population may be examined. Controlled experimental induction of lameness might be necessary to obtain such a group.

This study showed that open field activity and pressure mat analysis of kinetic parameters are able to detect the effects of analgesia in lame pigs. Although these methods provide no direct measures for pain, they may be useful as additional outcome parameters to assess pain and the effects of analgesia in pigs. A battery of tests, including traditional reflexive hypersensitivity as well as behavioural outcomes such as adaptive postural changes, changes in spontaneous behaviours, motivational aspects of pain and pain relief and emotional changes may be needed to fully elucidate the effects of current and new analgesics (Cobos and Portillo-Salido, 2013). Open field test activity and pressure mat analysis may provide research tools to study this topic.

Conclusions

The administration of 0.04 mg/kg buprenorphine to clinically lame pigs resulted in a more symmetric gait and more activity in an open field, thus demonstrating the ability of these methods to detect the effect of analgesia. Although these methods do not allow direct measurement of pain, they may be useful as part of a battery of tests to establish the effect of interventions on lameness-associated pain, thus improving welfare in lame pigs.

Conflict of interest statement

The authors declare that they have no financial or non-financial competing interests.

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Supplemental material

Pilot experiment: the effect of buprenorphine on open field activity and kinetic pressure mat parameters in sound pigs

Background

The aim of the main experiment was to assess the sensitivity of two methods: pressure mat gait analysis and open field test to the effects of analgesia. Buprenorphine was chosen as the analgesic agent.

Intramuscular injection of buprenorphine (a potent analgesic acting as a mu-opioid antagonist) caused mildly increased activity levels in pigs (Hermansen et al., 1986). In a study comparing intramuscular buprenorphine to transdermal fentanyl, no effects of buprenorphine activity levels or time between major movements were noted (Harvey-Clark et al., 2000).

This pilot experiment was performed to rule out side effects of the buprenorphine treatment that would influence the readout parameters of our study (gait asymmetry, locomotor activity).

Twenty-four sound pigs were assigned to one of three groups, which were either treated with a low (0.012 mg/kg), medium (0.04mg/kg) or high (0.12 mg/kg) dose of buprenorphine, injected i.m.

Materials and methods

Animals

24 clinically healthy Topigs 20 x Tempo pigs, aged 6 weeks, were selected based on general appearance and a basic clinical examination (including assessment of breathing, pulse, temperature, skin colour, mucous membranes and lymph nodes) from a commercial farm by a veterinarian and transported to the research facility of Utrecht University.

Housing

All pigs were housed at the research facility of Utrecht University in 3 pens with sawdust-covered solid concrete floors measuring 153 cm x 256 cm. Each pen contained 8 pigs. They were provided with 11 hours of light per day (7 a.m. to 6 p.m.) from both daylight and artificial lighting. Ambient temperature ranged from 22 to 24°C. All pigs had ad

libitum access to food (Groeiporco, De Heus Animal Nutrition BV), water and toys (plastic ball, metal chain).

Treatment

The analgesic used was Buprecare Multidosis (AST Farma BV), containing 0.3 mg buprenorphine hydrochloride per ml.

Data collection

The complete set of measurements (clinical exam, weighing, visuals scoring of gait, open field testing and pressure mat measurements) was performed on day 1. Twenty-four hours later, three groups of 8 pigs randomly divided over the pens each received an intramuscular injection with 0.012 mg/kg, 0.04 mg/kg, or 0.12 mg/kg buprenorphine.

1.5 hour after buprenorphine injection, the same battery of measurements was used as on the previous day. Measurements were taken on day 1 and 2 at the same time during the day.

First, data that could be collected without disturbing pigs were noted (breathing rate and skin color). Then, visual scoring of gait (Main et al., 2000) was performed. Heart rate, rectal temperature, mucous membranes, lymph nodes (to assess if the animals were clinically healthy) were examined and bodymass were measured.

Open field testing was performed in a pen (153 x 256 cm bordered by a 0.90 cm high wall) in a separate room. A video camera was mounted approximately 2.5 meters above the pen. The pig was transported to the pen using a cart, and was placed in the open field where testing immediately began. After 5 minutes the recording was stopped and the pig was removed from the pen. The pen was rinsed with clean tap water between each pig.

The pressure mat had an active sensor surface of 1.95 m × 0.32 m containing 16384 sensors with a sensitivity of 0.27-127 n/cm2 and a measuring frequency of 126 Hz (RSscan International NV) and was used in the same setup that was previously described by Meijer et al (2014a).

Pigs were guided one by one to the holding area. After one minute, the door leading to the runway was opened. Exploratory behaviour combined with candy rewards encouraged the pigs to walk across the runway. Three correct runs per pig were collected. A run was considered correct when the pig walked (gait confirmed by duty factor) across the runway without stopping, at a steady velocity and looking straight ahead.

On the second day, after data collection, the pigs were sedated with 2 mg/kg intramuscular Azaperone (Stresnil, Elanco Animal Health) and subsequently euthanized by intracardial injection of 200 mg/kg Pentobarbital (Euthanimal, Alfasan Diergeneesmiddelen BV). Gross pathology was performed at the Department of Pathobiology of the Faculty of Veterinary Medicine of Utrecht University,

Data analysis

Open field behaviour: Videos were scored using the purpose-built scoring program OBSERVE (Van Der Staay et al., 2009b). The screen was divided into 12 equal-sized squares and each time the pig crossed a line, this was scored by an observer. The number of times a pig reared (standing on hind limbs with front limbs against the pen wall) or jumped (attempt to escape from pen in which all four limbs are off the floor for a moment) was registered. The total amount of line crossings in 5 minutes was used as an index for locomotor activity.

Pressure mat: For the pressure mat data, claw strikes from the 3 valid runs were manually assigned to left fore (LF), right fore (RF), left hind (LH) and right hind (RH) limb. Four kinetic parameters were collected: Peak Vertical Force (PVF), Load Rate (LR), Vertical Impulse (VI) and Peak Vertical Pressure (PVP). The total left-right ASI (ASI) for each of these parameters compared the left limbs to the right limbs and was calculated using a formula modified from Oomen et al (2012):

$$ASI = \frac{(LF + LH) - (RF + RH)}{0.5 * \big((LF + LH) + (RF + RH)\big)} * 100$$

An ASI of 0 indicated perfect symmetry; the extreme values of -200 or +200 indicate non-weight-bearing lameness on either the left or right side. Absolute values for ASI were used for subsequent analysis. To assess the effect of dosage and treatment on the parameters, the difference between pre-and post-treatment (difASI for asymmetry indices and difOF for open field activity) were calculated.

Statistical analysis

Normality of the data was assessed using Q-Q plots and Kolmogorov-Smirnov test.

We first assessed if there was a difference between the three dosage groups before administration of buprenorphine (untreated baseline measurements). Secondly, we assessed if dosage group influenced difASI of the kinetic parameters, difOF and two specific OF behaviours (jumping, rearing). Differences between the three groups for normally distributed data were assessed using ANOVA, whereas differences between groups

for non-normally distributed data were assessed using the Kruskal-Wallis test. All data were analysed using SPSS version 20.0 for Windows (IBM) and R version 3.1.0 (R Foundation for Statistical Computing) and are presented as means ± SD. Statistical significance was set at $p < 0.05$.

Results

The 24 clinically normal pigs weighed 8.8 ± 1.3 kg. Clinical examination, visual scoring and necropsy did not reveal abnormalities in any of the piglets. Gait scores for all pigs were therefor 0, both pre-and post-treatment.

It was predicted that there would be no differences, in the measured parameters, between the randomly selected groups before treatment, this was confirmed (Table 1) in the statistical analysis [PVF: $\chi^2(2)=1.815$, $P=0.404$, LR: $\chi^2(2)=4.865$, $P=0.088$, VI: $F(2,21)=1.273$, $P=0.301$, PVP: $F(2,21)=0.468$, $P=0.633$ and open field crossings: $F(2,21)=0.743$, $P=0.488$].

No statistically significant differences were found after each of the three buprenorphine treatments (Table 2) [difASI PVF: $F(2,21)=1.220$, $P=0.315$, difASI LR: $F(2,21)=0.819$, $P=0.454$, difASI: VI ($\chi2(2)=1.095$, $P=0.578$, difASI PVP: $\chi2(2)=1.750$, $P=0.417$, difOF $F(2,21)=0.698$, $P=0.509$, number of times rearing χ^2 $(2)=4.174$, $p=0.124$ number of times jumping χ^2 $(2)=4.174$, $p=0.124$]. However, excitation (rearing, jumping against pen wall) was seen in 2 pigs after the highest dose (0.12mg/kg). The intermediate dose of (0.04mg/kg) was chosen for our main study.

Discussion

This pilot experiment revealed that the three doses of buprenorphine did not affect behaviour in healthy pigs, but some mild excitation was observed after treatment with the highest dose. Gait symmetry was not affected by buprenorphine treatment.

The doses used here (0.012, 0.04 and 0.12 mg/kg) reflect the wide range (0.005-0.12 mg/kg) of recommended dosages found in literature (Hermansen et al., 1986; Kaiser et al., 2006; Roughan and Flecknell, 2002; Swindle and Smith, 2013). Duration of action in these studies ranged from 6 to 24 hours. Onset of action ranged from 30-60 minutes (Hermansen et al., 1986). In the present study, pigs were tested 1 ½ hour after administration of buprenorphine, and it took less than 4 hours to complete the full set of measurements, so analgesia was expected to last throughout the measurements.

No significant changes were found in the measured behavioural parameters after i.m. administration of Buprenorphine at doses 0.12mg/kg body mass. This is consistent with

the findings of Harvey-Clark et al. (2000). However, 0.12mg/kg may produce increased behavioural activity as seen in the responses of 2 out of 8 pigs receiving the high dose in this study and as reported previously (Hermansen et al., 1986; Malavasi, 2005).

None of the sound pigs had a completely symmetric gait. Previous research in pigs showed that sound animals do display some asymmetry (Karriker et al., 2013; Meijer et al., 2014a, 2014b) and there is some evidence for limb dominance in kinematic analysis of total support moments in dogs (Colborne et al., 2008). We used each pig as its own control thus eliminating possible individual limb loading preference.

Ataxia is a known side effect of some opiates, and ataxia might result in an increased asymmetry of gait. Ataxia is, however, extremely rare after buprenorphine administration (Cowan et al., 1977). We did not find any treatment effect on asymmetry in the present pilot experiment.

Conclusions

In sound pigs, gait symmetry or open field activity was unaffected by intramuscular buprenorphine regardless of dose. Slight excitation was seen in high doses (0.12 mg/kg). Therefore, we chose the medium dose of 0.04 mg/kg for the main experiment.

5

The effect of NSAIDs on gait in experimentally induced chronic osteoarthritis in growing pigs

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In preparation

Abstract

Lameness is a common health issue in the pig-farming industry that causes both severe welfare issues for affected pigs as well as economic impact for farmers. A large proportion of these issues are either a direct or an indirect consequence of locomotor pathology, inflammation and pain. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), targeting both the inflammatory process as well as pain are expected to ameliorate the underlying condition as well as its consequences and thus, provided administration in an appropriate dosing scheme, may be an effective intervention in affected animals. However, limited information on the effect of NSDAIDs, particularly on chronic lameness in weaned piglets is available.

Lameness is often caused by joint disease, such as osteoarthritis. Experimentally induced progressive osteoarthritis was chosen to model both acute and chronic pain due to lameness. Intra-articular injection of Monosodium-iodoacetate (MIA) is a well-known method to experimentally induce osteoarthritis in rats and mice, but has not yet been used in piglets.

MIA or 0.9% saline solution was injected into the left carpal joint of a group (n=40) of 7-week-old piglets. Objective kinetic pressure mat analysis was performed before induction of lameness and on day 1, 3, 7, 14, 28 and 56 after induction. Asymmetry indices comparing left limbs to right limbs were calculated for each kinetic parameter and were used as a measure for lameness. In addition, all animals were visually scored for lameness using a 6-point lameness scoring system developed for finishing pigs. Scoring was performed by two experienced observers who were blinded for treatment and day. Video recordings taken prior to pressure mat runs in a dedicated stable area before and 1, 3, and 28 days after injection were used to perform visual scoring.

Post-mortem macroscopy and microscopy of the piglets confirmed structural changes in 18 of the 20 MIA-injected joints (90%). Functionally, MIA induced a fairly stable lameness in piglets. Due to decreased weight-bearing in the left front limb, mean asymmetry indices of pressure mat parameters were reduced in the MIA-treated animals from day 1 to day 28. At day 56, no significant differences were observed anymore. Mean visual lameness scores showed a peak at day 1, and after a slight transient improvement lameness increased again.

Visual lameness scoring and asymmetry in parameters detected by the pressure mat were correlated. However, when specific cutoffs were set in the data analysis program used for identifying individual animals that were injected with MIA, differences between both methods of lameness detection became obvious. Although lameness was not observed by visual scoring on day 3, asymmetry indices did detect lameness in several

MIA-injected animals at this timepoint. Overall, pressure mat parameters did appear to be more sensitive to MIA-induced gait abnormalities than visual scoring, however this may come at the cost of some false-positive observations.

Intra-articular injection of MIA induces both structural changes (observed at necropsy) and functional changes (asymmetric weight bearing and higher mean visual lameness scores) in joints of weaned piglets. The identification of joint pathology in individual animals by functional measures however remains a challenge. Meloxicam administered once daily at the registered dose did not affect the observable and measureable signs of lameness.

Introduction

Osteoarthritis is a common cause of lameness in pigs and may be caused by infectious agents or by osteochondritic lesions that provoke a sterile osteoarthritis (Jensen and Toft, 2009). Regardless of its causes(s), osteoarthritis is an economical problem, due to the costs of treatment or premature culling of lame animals and reduced daily weight gain (Jensen et al., 2012, 2007). More importantly, it causes lameness and therefor welfare issues in affected animals (Anil et al., 2009; Jensen et al., 2012). These welfare issues may be pain, a reduced mobility, and as a consequence a reduced ability to exhibit species-specific behaviors, to maintain social status within the group and to reach feeding and drinking stations (Anil et al., 2009).

A potential intervention to mitigate these welfare issues associated with osteoarthritis is the use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). These drugs have both anti-inflammatory and analgesic properties and are therefore expected to benefit pigs that suffer from osteoarthritis. Meloxicam is a NSAID which is currently licensed in a once a day dose of 0.4 mg/kg PO or IM in "pigs with conditions affecting the locomotor system", to be repeated the next day at the discretion of the farmer ("EMA, European public assessment reports: veterinary medicines," 2015). An earlier study looking at the effect of meloxicam on experimental inflammatory markers in young pigs suggests that this dose may be insufficient to adequately inhibit inflammation and that penetration into inflammatory exudate is limited (Fosse et al., 2011). Several studies have been performed to assess the clinical effectiveness of NSAIDs in lame pigs. Some of these studies have used visual gait scores as readout parameters (Friton et al., 2002; Mustonen et al., 2011), while others used objective methods such as kinetic measurements (Pairis-Garcia et al., 2015) or mechanical threshold testing (Karriker et al., 2013; Mohling et al., 2014; Nalon et al., 2013; Tapper et al., 2013). These studies have either used naturally-occurring lameness or experimentally induced lameness, but all have focused on acute lameness, and have only assessed part of the pain experience.

Measuring pain in animals is challenging, and no single method up to date has been able to detect or quantify all different aspects of pain (Mogil, 2009). Pain must therefore be assessed on several levels (Cobos and Portillo-Salido, 2013; Gregory et al., 2013; Mao, 2012; Mogil, 2009). Most studies of pain management interventions in (experimental) animals focus on the sensory-discriminative component of pain or rely on assessor based composite weighted pain scoring systems and are known to suffer from suboptimal sensitivity and objectivity (Hansen, 2003). This has hampered the effort to establish clinically effective pain management interventions (Cobos and Portillo-Salido, 2013; Mogil, 2009).

In the present study therefore, we used a multilevel approach to measure pain due to experimentally induced osteoarthritis. To assess and quantify the *sensory-discriminative component* of pain, mechanical threshold testing using a limb mounted actuator was performed. Gait analysis, in the absence of ankylotic joint disease on pathology, assessed and quantified the *affective-motivational aspects of joint pain* while the *cognitive-evaluative component of pain* was assessed using a place aversion/place escape paradigm.

We used Monosodium-Iodo-Acetate (MIA) induced osteoarthritis in the pig as model for assessing the effect of Meloxicam in the registered dose for prolonged duration on all three pain components. Intra-articular Monosodium-Iodo-Acetate (MIA) is reported to consistently produce osteoarthritic joint changes which progress into a chronic osteoarthritis associated with clinical lameness and nociceptive changes in rodents. This allows the study of both the acute and chronic osteoarthritic phase within the same subject. Although to date no MIA-induced experimental osteoarthritis in pigs are reported, we conducted a pilot study indicating that a dose of 20 to 40 mg intra-articular MIA in the carpal joints of young pigs at 10 days post injection results in typical MIA associated microscopic changes seen in rodents (data not shown).

In the current study we followed animals over time, in order to assess both the acute and chronic phase of osteoarthritis. The effect of meloxicam on three different methods of pain assessment was investigated, and the correlation between these methods was determined. In this chapter the structural and functional changes caused by intra-articular injection of MIA in the left intercarpal joint, and the effect of meloxicam on these functional changed are presented. Structural changes were assessed by postmortem examination of joints, while functional changes were assessed by both the pressure mat analysis and visual scoring are presented in this chapter to investigate *affective-motivational aspects of pain*, the effect of the experimental intervention on other aspects of pain (the sensory-discriminative component pain provoked by a mechanical threshold test and the cognitive-evaluative component of pain assessed using a place aversion/place escape paradigm)is currently being investigated.

Materials and methods

The study was reviewed and approved by the local ethical committee of Utrecht University (no. 2014.I.11.085, date of approval 17 December 2014), The Netherlands, and was conducted in accordance with the recommendations of EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering. A rescue protocol was in place for severely lame animals, consisting of 10 μg/kg buprenorphine intramuscular (Buprecare Multidosis, AST Farma BV, The Netherlands, 0.3 mg/ml) every

12 hours when required. Rescue analgesia was administered to 8 animals that received a MIA injection, approximately 2 hours after recovery from anesthesia on day 0.

Animals

Fourty Topigs 20 x Pietrain cross piglets were selected at 28 days of age from the Utrecht University teaching farm. Five litters were used, and from each litter 8 pigs were selected. They underwent a general clinical examination and visual assessment of locomotion. Only healthy, sound piglets were selected. When more than 8 suitable animals were present in a litter, the heaviest animals were chosen. Due to logistical reasons, animals were selected in two batches that were 3 weeks apart. The selected piglets were transported to the research stables of the Behavior and Welfare group. To minimize fighting and possible associated injuries, pigs were housed with their littermates and were not mixed with unfamiliar pigs.

Housing

Piglets were housed per 8 littermates in pens with solid concrete floors and deep straw bedding. They were provided with a covered nest area. The roof of the nest area had rubber flaps hanging down and could be lowered to provide extra shelter during the first weeks. Each nest area had one heat lamp per 2 $m^2$and the floor was covered with a rubber mat with straw bedding on-top. Enrichment consisting of metal chains, balls, and rubber chewing sticks was provided for the entire duration of the study.

Water was available *ad libitum* through a drinking nipple. The pigs were fed commercial standard food for growing pigs (Stimulans, De Heus, The Netherlands) *ad libitum* in a large food trough. The piglets were weighed twice a week during habituation and in the first two weeks of testing, and once a week in the following weeks. A radio was playing in the stable day and night, including during testing.

Handling and Habituation

Piglets were handled daily by the researchers and were habituated to the different experimental set-up in progressively smaller groups, until animals were able be alone in the experimental set-up. In order to minimize the time needed to collect successful trots for the videotaped open space locomotion as well as the for the pressure mat runway, the pigs were trained before the start of the experiment using positive reinforcement or "clicker training" with milk chococlate treats (M&M's®, Mars Chocolate, Veghel, Netherlands). Subsequently, normal exploratory behavior across the runway was shaped until each pig was able to trot down the runway in a straight line at constant speed without stopping.

Experimental design

Piglets were randomly assigned to one of four groups:

- MIA treated, no NSAID ("OA")
- MIA treated, NSAID ("OAN")
- Saline treated, no NSAID ("SC")
- Saline treated, NSAID ("SCN")

Visual scorings and pressure mat analyses were performed on video recordings and pressure mat measurements collected on several timepoints (Table 1).

Intra-articular injection

To ensure proper intra-articular injection technique, injections were practiced with toluidine blue on post-mortem material of pigs of the same size as the control and experimental animals.

At day 0, the day of the experimental induction of lameness, animals were sedated with an intramuscular injection of dexmedetomidine (15 µg/kg, Dexdomitor 0.5 mg/ml, Orion Pharma, Finland). When sufficiently sedated, an i.m. injection of ketamine (10 mg/kg, Narketan 10, Vétoquinol S.A., France) and midazolam (0.5 mg/kg, Midazolam Actavis 5 mg/ml, Actavis Group PTC, Iceland) was given to induce general anaesthesia. After 5 minutes the left intercarpal joint was prepared for aseptical surgery by shaving and disinfecting with 70% alcohol and chlorhexidine (Hibisol, Regent Medical Ltd., UK). Pigs received an intra-articular injection with either 0.25 ml MIA (80 mg/ml solution, sodium iodoacetate BioUltra 98%, Sigma Aldrich, USA) or 0.25 ml of sterile NaCl 0.9% (B. Braun Melsungen AG, Germany). The left carpus was slightly bended in a 100° to 110° angle, under gentle distention, and the needle was inserted medially, 2 to 3 mm deep, into the intercarpal joint. The pigs recovered from anaesthesia in a separate area, with at least one caretaker present at all times. Soft flooring was provided.

NSAID treatment

Meloxicam (0.4 mg/kg, Metacam 15 mg/ml oral suspension for pigs, Boehringer Ingelheim Vetmedica GmbH, Germany) was administered orally once a day between 16:00 and 19:00 hours in approximately 10 ml of apple sauce through a 20 ml syringe (Omnifix, B. Braun Melsungen AG, Germany) to the pigs in the OAN and SCN groups. Pigs in the OA and SC group received approximately 10 ml of apple sauce without meloxicam in the same way at the same time.

Table 1. General overview of the experimental procedures, including analgesia.

Day	Procedures	Analgesia
-21	Selection of litters and piglets Weaning and transportation to experimental facility	
-21 to -1	Training and habituation (to human presence and being alone in the different test set-ups)	
-1	Baseline measurements: • Pressure mat measurements • Video recordings for visual scoring	
0	Intra-articular injection: • MIA (20 animals, 5 ♂, 15 ♀) • Saline (20 animals, 10 ♂ ,10 ♀)	Rescue analgesia (buprenorphine) for 8 animals in the MIA-injected group
1	Pressure mat measurements Video recordings for visual scoring	NSAID (Meloxicam) once daily: • 10 MIA-injected animals (4 ♂, 6 ♀): OAN • 10 saline animals (4 ♂, 6 ♀): SCN
3	Pressure mat measurements Video recordings for visual scoring	
7	Pressure mat measurements	
14	Pressure mat measurement	
21	Pressure mat measurement	
28	Pressure mat measurements Video recordings for visual scoring	
56	Pressure mat measurement	
63	Euthanasia	

Measurements

Weighing

Animals were weighed twice a week during habituation and in the first two weeks of testing, and once a week in the following weeks.

Visual scoring

Two experienced observers, one Board-certified porcine health specialist/ECPHM diplomate (AvN) and one ECPHM resident (LDP), visually scored all pigs for lameness. They were blinded for both treatment and experimental day. To make this possible, they scored movie clips of the pigs. Each animal was individually filmed for 2-3 minutes before treatment and on day 1, 3 and 28. Animals were encouraged to stand, walk and trot and were filmed from all sides. Observers were allowed to watch the movies as often as they needed to reach a decision on the score, but they could not use slow motion or freeze the movie at any time.

Visual scoring was performed according to a protocol modified from Main et al (Main et al., 2000). Since the original protocol also incorporates features that assess behavior within the group and this could not be videotaped without unblinding the observers for pig ID, all descriptors that incorporated behavior within the home pen were removed from the original protocol, resulting in the modified protocol described in Table 2.

Pressure mat analysis

The pressure mat, a Footscan® 3D Gait Scientific 2 m system (RSscan International, Olen, Belgium) with an active sensor surface of 1.95 m × 0.32 m containing 16384 sensors (2.6 sensors per cm^2), with a sensitivity of 0.27-127 n/cm^2 and a measuring frequency of 126 Hz was connected to a laptop with dedicated software (Footscan Scientific Gait 7 gait 2^{nd} generation, RSscan International, Olen, Belgium). Before each measuring session, the footscan was calibrated according to the manufacturer's specifications using a person weighing 62 kg. The mat was mounted flush with a 40 cm x 1000 cm runway. A holding pen with a trap door was located at both ends of the runway. The entire runway was covered with a rubber mat (5 mm thick, shore value 65° ± 5).

Table 2. Visual scoring protocol modified from Main et al (Main et al., 2000). **Bold type** identifies the defining criteria that must be present to assign a score. Normal type shows supporting criteria that are useful for assigning a score.

Lameness score	Standing posture	Gait
0	Pig stands squarely on 4 legs.	**Even strides**. Caudal body sways slightly while walking. Pig is able to accelerate and change direction rapidly.
1	As for score 0.	**Abnormal stride length** (not easily identified).**Movements no longer fluent** (pig appears stiff). Pig still able to accelerate and change direction.
2	**Uneven posture.**	Shortened stride. Lameness detected. Swagger of caudal body while walking. No hindrance in pig's agility.
3	**Uneven posture. Will not bear weight on affected limb** (appears to be standing on toes).	Shortened stride. Minimum weight-bearing on affected limb. Swagger of caudal body while walking. Will still trot and gallop.
4	**Affected limb elevated off floor**. Pig appears visibly distressed.	**Pig may not place affected limb on the floor while moving.**
5	Will not stand unaided.	**Does not move.**

On measuring days, pigs from one pen were placed in a holding area adjacent to the runway. The pig in the runway was able to hear and smell its penmates, although it could not see them. Pigs were let into the runway one by one and tested in the order they presented themselves in. Two people at either side of the runway operated the trap doors and used clickers and M&M's to reward the pigs. A third investigator operated the software and judged each run on the following criteria: Pigs had to trot the entire length of the runway at a visually steady pace in a straight line and looking straight ahead. Runs

that fulfilled these criteria were saved and the process was repeated until 4 runs meeting these criteria were collected.

Claw strikes were manually assigned to left front (LF), right front (RF), left hind (LH) and right hind (RH) limbs using the Footscan software. Peak vertical force (PVF (N)) , Load rate (LR (N/s)), Vertical Impulse (VI (Ns)) and Peak Vertical Pressure (PVP (N/cm^2)) were selected from the measures calculated by the program and used to derive a left-right asymmetry index comparing left limbs to hind limbs (contralateral total, CLT) using a formula modified from Oomen et al (Oomen et al., 2012):

$$CLT = \frac{(LF + LH) - (RF + RH)}{0.5 * \big((LF + LH) + (RF + RH)\big)} * 100$$

Additionally, a left-right asymmetry index comparing just the front limbs (contralateral front, CLF) was calculated using the following formula:

$$CLF = \frac{LF - RF}{0.5 * (LF + RF)} * 100$$

An ASI of 0 indicated perfect symmetry and the extreme values of -200 or +200 indicated non-weight-bearing lameness on either the left or right side.

Euthanasia

Sedation and anaesthesia of the animals prior to IV pentobarbitone overdose was induced in the same way as described for the intra-articular injections. As soon as a sufficient level of general anaesthesia was reached, IV access (ear vein, jugular of superficial abdominal vein) was secured using an IV cannula and the pigs were euthanized by intravenous injection of 50 ml of Pentobarbital (Euthanimal, Alfasan, Woerden, The Netherlands, 400 mg/ml)). After euthanasia, the pigs were transported to the Department of Pathobiology of the Faculty of Veterinary Medicine of Utrecht University.

Histopathology

Within hours of euthanasia, a full necropsy including opening of left and right carpal joints, shoulder joint, elbow joints, knees and tarsal joints was performed. Samples of ca. 4mm thick were taken of both radiocarpal joints using a K430 band saw (Kolbe, Germany; blades Munkfors, Sweden). Furthermore samples were taken of both kidneys, stomach, duodenum, jejunum, ileum, caecum and colon. These samples were placed in 10% neutral buffered formalin and stored at room temperature until sufficiently fixated. After fixation the samples of the joints were decalcified in 10% ethylene-diamine-tetra-acetic acid (EDTA). The degree of decalcification was evaluated every week by testing how easy the

tissue could be bend and whether the tissue could be penetrated by a sharp metal pin. When the tissue was not decalcified sufficiently samples were put in a new EDTA solution. Decalcification time varied between 2 and 15 weeks. Hereafter the samples were embedded in paraffin, cut into sections of 3 micrometer, stained with haematoxylin and eosin (HE)and evaluated by light microscopy (Olympus BX-45, Zoeterwoude, The Netherlands).

Statistical analysis

Average daily weight gain between the four groups was assessed using analysis of variance (ANOVA).

A linear mixed effects model was used to evaluate the effect of day and group (OA, OAN, SC, SCN) as fixed factors on CLF of PVF, LR, VI and PVP. Pig was considered as random effect. To accommodate for differences in variability between groups a variance function was added to the model. If appropriate, the model was further reduced by combining the SC and SCN group into one new group ("Control"). Model selection was based on Akaike's information Criterion (AIC), and the appropriateness of the models was evaluated by a visual inspection of graphs and residues for normality and homoscedasticity.

Agreement between two observers assigning visual scores was assessed using Cohen's unweighted ƙ.

All analyses were performed using R Statistical software version 3.1.2 (R Development Core Team, 2008) with package nlme (Pinheiro et al., 2015). Statistical significance was set at $P<0.05$. Unless indicated otherwise, results are presented as mean +/- SD.

Results

Average daily weight gain of the piglets during the experiment was 705 ± 68 gram and did not differ between groups.

Pathology

At pathology, none of the animals in the control groups showed signs of osteoarthritis. However, within the articular cartilage of some of the control carpi areas of necrosis were present characterized by shrunken and hypereosinophilic chondrocytes and loss of chondrocytes, compatible with osteochondrosis latens.

Two animals (ID nr. 12 and 25, both from the MIA group) that had received MIA did not show lesions in the injected joint and were therefor excluded from the analysis. Macroscopically, the joint surfaces of the radiocarpal joints of the injected carpi of the

other MIA-treated animals were severely irregular and dull with multifocal red discolorations. Of some of the affected joints the synovial membranes were mildly to moderately thickened. None of the affected joints showed an increase of synovial fluid. Histologically, the joint surfaces were moderately irregular. The articular cartilage lost its hyperchromatic basophilic staining, and multifocally chondrocytes were either shrunken and hypereosinophilic, or not present at all (necrosis). Occasionally there was formation of fissures and cystic spaces in the necrotic cartilage. These necrotic areas were multifocally surrounded by vital cartilage in which clusters of hyperplastic chondrocytes (chondrones) were visible. Multifocally the subchondral bone was replaced by hypereosinophilic amorphic material in which shrunken, hypereosinophilic, necrotic osteocytes were present. Within the bone marrow surrounding these areas mildly increased amounts of osteoclast and moderately increased amounts of osteoblast were seen. Multifocally both cartilage and subchondral bone were replaced by fibrovascular tissue.

Pressure mat analysis

In the saline-treated group, CLF of all four parameters (PVF, LR, VI and PVP) was lower on day 1 compared to the baseline measurement. The difference was comparable for all parameters (fig. 1-4, table 3).

The MIA-treated group that did not receive NSAIDs showed lower CLF compared to the baseline measurement on day 1 for LR and VI. On day 3 CLF of PVF, LR and VI was lower. CLF of all four parameters was lower on day 14 and 28. On day 56, only CLF of PVP returned to baseline level (fig. 1-4).

The MIA treated group that received NSAIDs showed lower CLF for all four parameters on day 1, 3, 7, 14 and 28. On day 56, only CLF of PVP still was lower compared to baseline level (fig. 1-4).

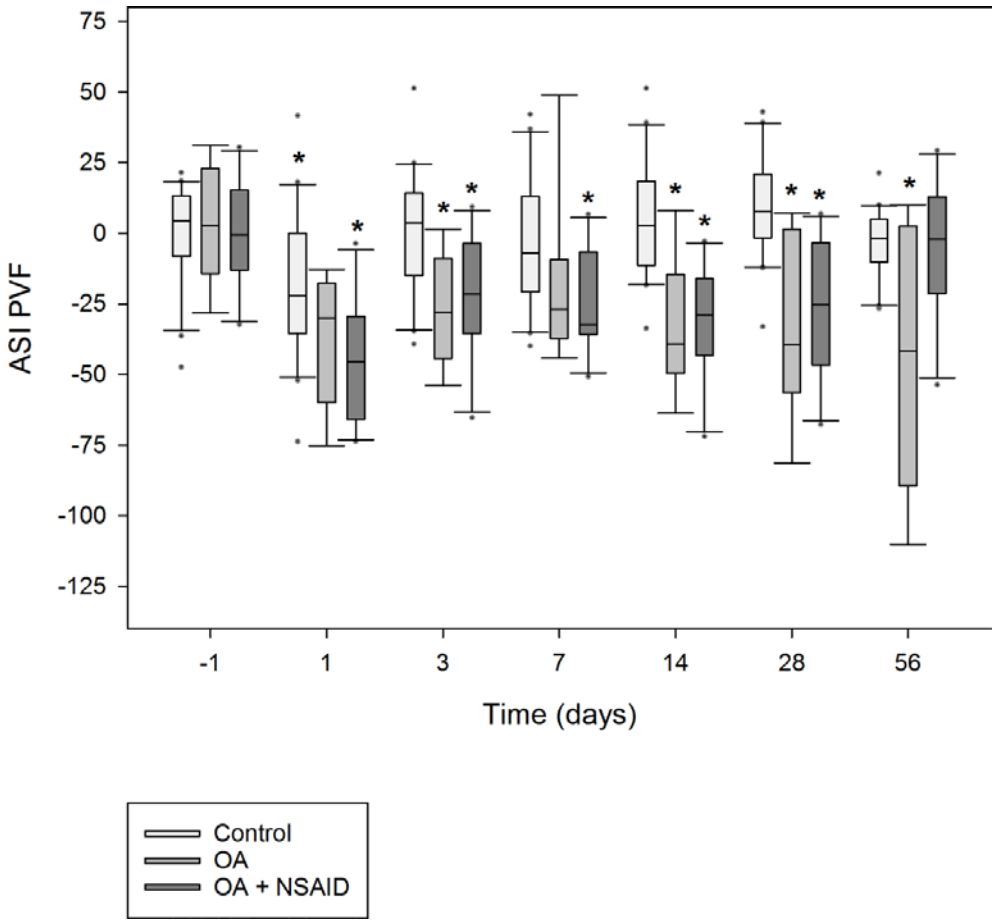


Figure 1. Boxplots for asymmetry indices (ASI) of PVF (Peak Vertical Force) of saline-injected (white boxes), MIA injected (light grey boxes) and MIA-injected and NSAID-treated (dark grey boxes) over time. Lower values suggest less weight-bearing on the left limbs. * indicates a significant difference from values at day -1.

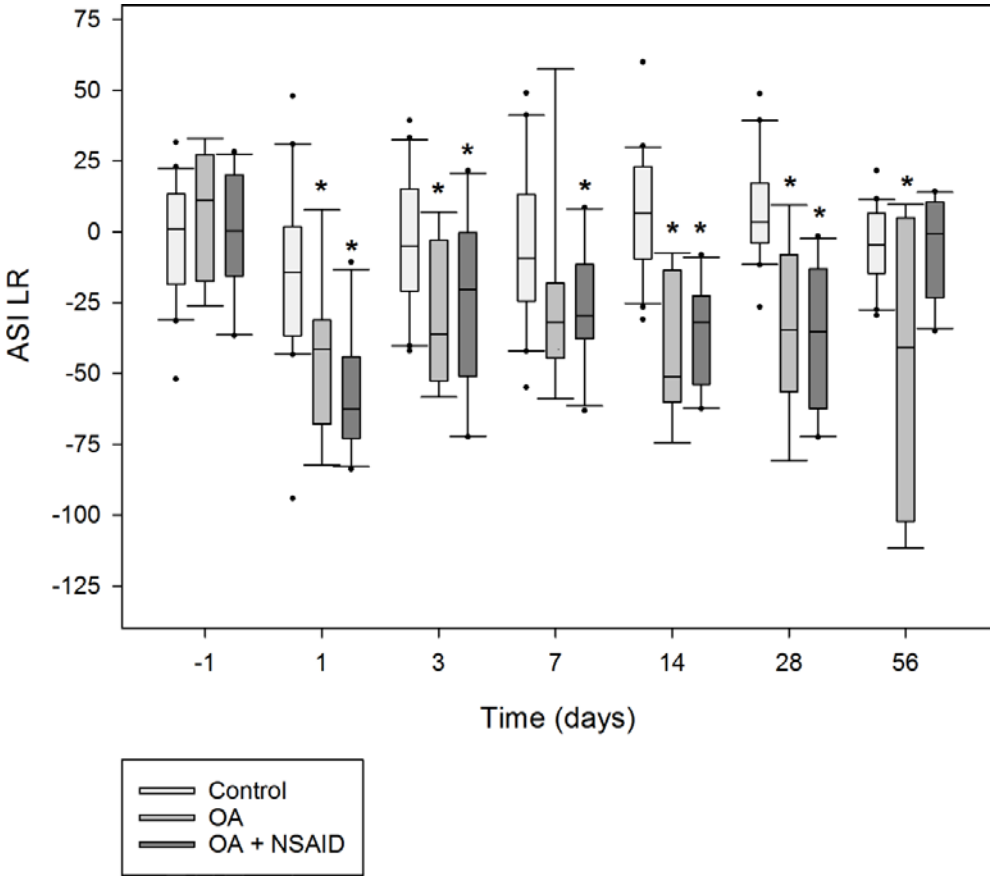


Figure 2. Boxplots for asymmetry indices (ASI) of LR (Load Rate) of saline-injected (white boxes), MIA injected (light grey boxes) and MIA-injected and NSAID-treated (dark grey boxes) over time. Lower values suggest less weight-bearing on the left limbs. * indicates a significant difference from values at day -1.

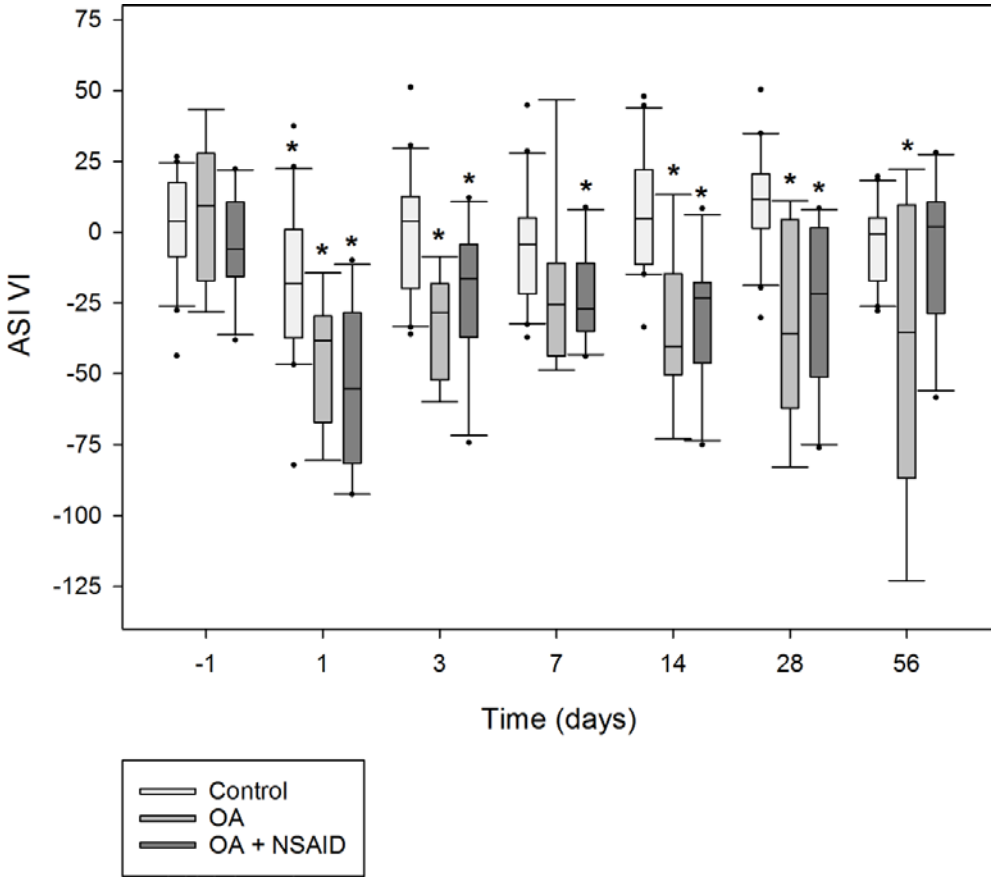


Figure 3. Boxplots for asymmetry indices (ASI) of VI (Vertical Impulse) of saline-injected (white boxes), MIA injected (light grey boxes) and MIA-injected and NSAID-treated (dark grey boxes) over time. Lower values suggest less weight-bearing on the left limbs. * indicates a significant difference from values at day -1.

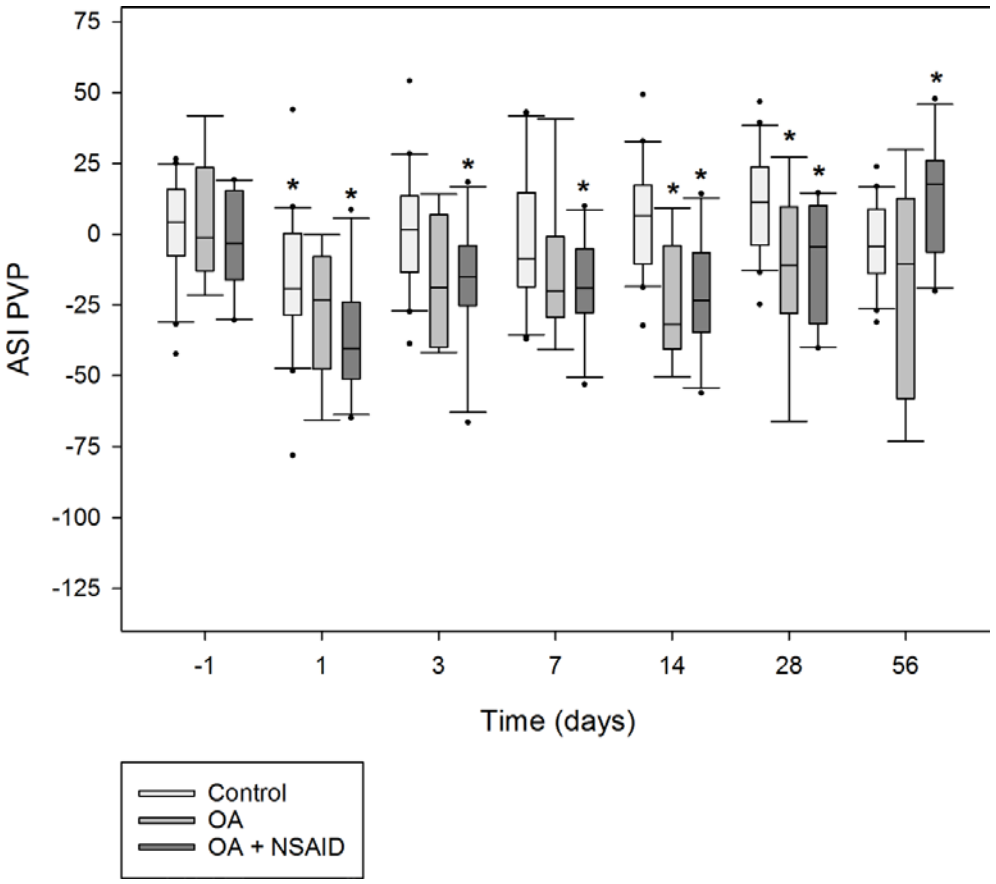


Figure 4. Boxplots for asymmetry indices (ASI) of PVP (Peak Vertical Pressure) of saline-injected (white boxes), MIA injected (light grey boxes) and MIA-injected and NSAID-treated (dark grey boxes)over time. Lower values suggest less weight-bearing on the left limbs. * indicates a significant difference from values at day -1.

Visual scoring

Due to technical difficulties, two movies were not available to score. These were a movie of one pig from the OAN group on the baseline measurement, and a movie of one pig from the OA group on day 3 (table 4). There was fair agreement between the two observers, κ=0.342, p<0.005.

Visual scores correlated with all pressure mat parameters CLTs, although the correlations were low (CLT PVF: r=-0.243 p<0.05,CLT LR: r=-0.257 p<0.05, CLT VI: r=-0.301 p<0.05, CLT PVP: r=-0.207 p<0.05).

Mean visual scores for MIA animals peaked at day 1 for both observers, and after an initial drop started climbing again. Observer B tended to assign lower scores than rater A, as can be seen in fig. 5.

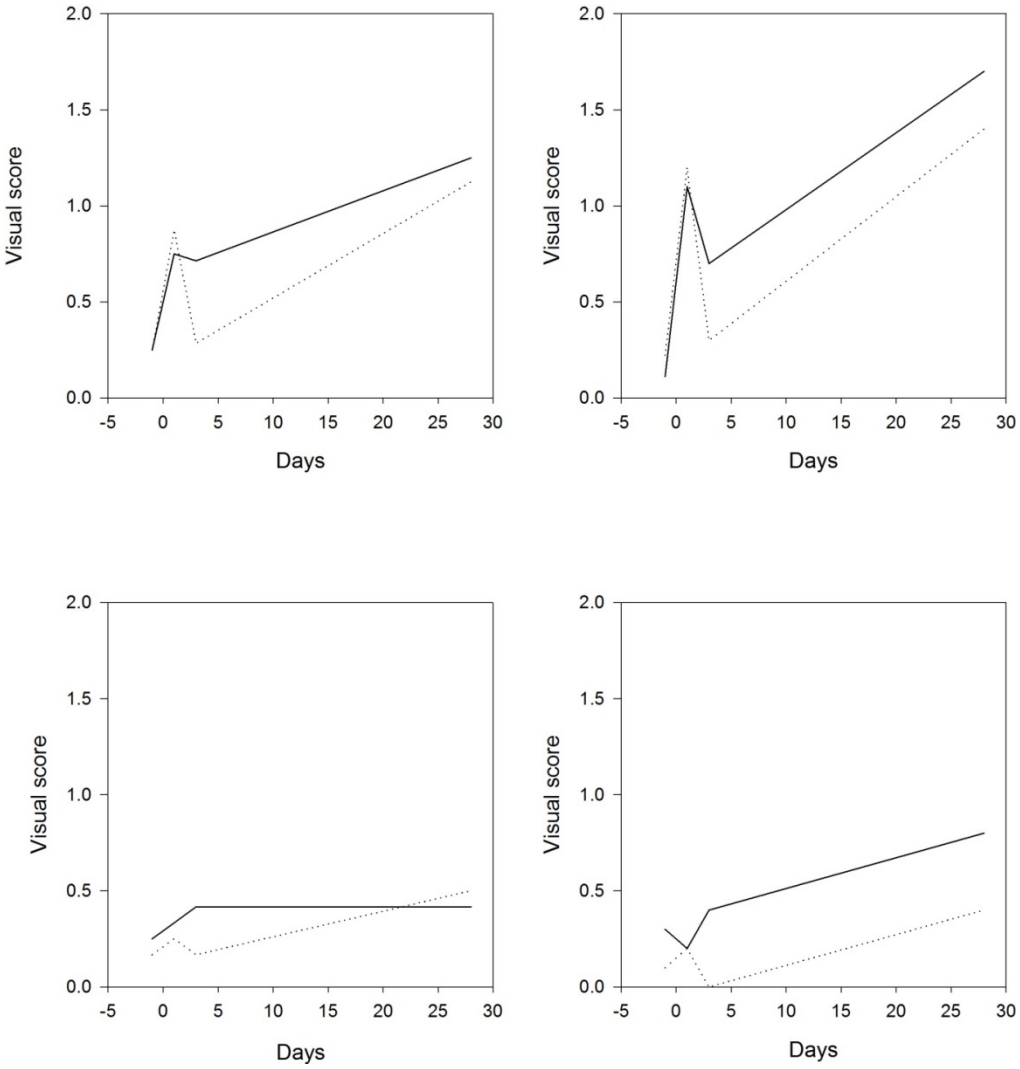


Figure 5. Visual scores of MIA (top left panel), MIAN (top right panel), SC (lower left panel) and SCN (lower right panel) assigned by observer A (solid line) and observer B (dashed line). Higher values represent more severe lameness.

When cutoff values for visual lameness scores were set at scores higher than 1, and cutoff CLT of pressure mat parameters at >25 or <-25, at baseline a few animals that were

visually sound exhibited asymmetry on some, or all, of the pressure mat parameters (table 3). On day 1, although none of the saline-injected animals were visually lame, asymmetry was present in some of these animals. In the MIA-injected group there were several individuals that were visually observed lame by observer B, although observer A only identified one animal as lame. Asymmetry indices identified more of the MIA-injected animals as lame. On day 3, none of the MIA injected animals was visually lame, although there were several individuals that exhibited asymmetry on the pressure mat. On day 28, lameness was present in more MIA-injected animals on both visual inspection and pressure mat analysis.

Table 3. Lameness, identified by pressure mat analysis and observers are indicated with a dot. Identification was based on cutoff values below -25 for CLT, or visual scores above 1 for observers. PVF= asymmetry index of Peak Vertical Force, LR = asymmetry index of Load Rate, VI=asymmetry index of Vertical Impulse and PVP=asymmetry index of Peak Vertical Pressure), obs. A = observer A and obs. B= observer B. SC = Saline-treated animals (N=10), SCN = Saline treated animals, received NSAIDs (N=10), OA = MIA-treated animals (N=8), OAN = MIA-treated animals, received NSAIDs (N=10). X: video not available.

	Group	SC										SCN										OA								OAN							
Baseline	CLT PVF					•									•											•											
	CLT LR					•								•	•							•				•							•				
	CLT VI					•									•							•															
	CLT PVP					•									•																						
	Observer A																													X							
	Observer B																													X							
Day 1	CLT PVF		•		•	•	•		•															•	•		•		•	•	•	•		•		•	
	CLT LR		•			•	•		•	•														•	•		•		•	•	•	•	•	•	•	•	
	CLT VI		•			•	•		•									•				•		•	•			•	•	•	•	•	•		•		
	CLT PVP		•		•	•			•															•	•			•	•	•		•	•		•		
	Observer A																														•			•				
	Observer B																									•								•	•	•	•	
Day 3	CLT PVF																				•						•	•						•	•	•	•	
	CLT LR																				•	•	•	•				•						•	•	•	•	
	CLT VI																				•		•				•	•	•					•	•	•	•	
	CLT PVP										•										•							•							•		•	•	
	Observer A																								X													
	Observer B																								X													
Day 28	CLT PVF								•				•								•	•			•		•			•				•	•	•		
	CLT LR			•					•				•								•	•		•	•					•		•			•	•		
	CLT VI								•			•	•								•	•			•		•			•					•			
	CLT PVP								•				•								•						•			•					•			
	Observer A																								•		•			•		•			•	•	•	
	Observer B																							•	•		•			•		•			•	•	•	

Discussion

Naturally-occurring animal models are often considered the best reflection of the actual disease in both humans and animals. They have, however, some limitations. Especially in larger animals, disease development and – progression may take months to years, may affect multiple sites in the body and in many animals the disease will never develop at all (Teeple et al., 2013).

In this study, we used MIA as a controlled experimental method to induce osteoarthritis. MIA interferes with cartilage metabolism by inhibition of chondrocyte glycolysis (Kalbhen DA, 1987; van der Kraan et al., 1989). It induces dose-dependent changes in chondral and subchondral tissue in rats, and with higher doses (0.3 and 3 mg in 50 µl) results in a decreased spontaneous locomotor activity and secondary progressive long-term loss of spontaneous mobility (Guingamp et al., 1997). It is difficult to compare doses used in joints of different species, especially when they differ in size. Therefore, the dose we administered to our pigs (20 mg in a 80 mg/ml solution) was based on a pilot study using three doses: 10 mg, 20 mg or 40 mg (data not presented). Both functional impairments and histological evidence of osteoarthritis development were seen in the 20 and 40 mg dose, however, changes in the 40 mg dose were more severe and expected to progress to unacceptable levels of lameness and possibly ankyloses. The 20 mg dose was therefore chosen for this study.

In the present study, MIA injection failed to induce macroscopic or microscopic signs of osteoarthritis in two animals. Although injection technique was practiced extensively on cadavers, it is possible that the MIA was not injected in the joint. No signs of extra-articular injection such as local swelling, redness or heat were observed in these two animals. In the 18 other animals, Mia induced structural macroscopical and histological changes that were comparable to those seen in other species (Combe et al., 2004; Guingamp et al., 1997; Guzman et al., 2003) and that were characterized by chondral erosions, chondrocyte necrosis, the formation of chondrones and exposure of subchondral bone.

Functional characteristics of MIA-induced osteoarthritis were assessed by pressure mat analysis and visual scoring. It has previously been shown that decreased weight-bearing is a useful measure for lameness in both experimentally-induced (Karriker et al., 2013; Pairis-Garcia et al., 2015)and naturally-occurring (Meijer et al., 2015, 2014) lameness in pigs. The effects of MIA on weight-bearing in pigs have not been described before.

We used two ASIs to describe the changes in weightbearing. CLF, comparing the two front limbs, was expected to be the most sensitive method to assess the effects of MIA injection and NSAIDs, since it only takes into account the affected limb and the limb receiving the

majority of the redistributed weight (Meijer et al., 2014). However, in order to compare visual assessment of lameness to pressure mat analysis, we used the CLF which takes all four limbs into account. Observers that scored lameness in the pigs did not know which pigs were injected with MIA and in what limb, so an ASI taking into account all four limbs as well was considered to be the best comparison.

Mean weight-bearing asymmetry of MIA-injected animals, quantified by CLF of kinetic pressure mat parameters, decreased on day 1 and 3 and showed a transient improvement on day 7. In the OAN group this even resulted in CLFs that did not differ from baseline measurements. At day 14, weightbearing decreased again and did not fully recover for the remainder of the study. A bi-phasic decrease in weight-bearing was previously observed in rats injected with MIA in the knee joint (Pomonis et al., 2005). In the first phase, immediately after injection, rats showed a marked decrease in weight-bearing that was lowest at 4 days post-injection. By 7 days post-injection, this had almost entirely resolved, but at day 14 post-injection a second, less pronounced stage of decreased weight-bearing developed which did not resolve for the remainder of the study (28 days post-injection). This biphasic decrease in weight-bearing was also observed in rats by Fernihough et al (Fernihough et al., 2004). Ivanavicius et al (Ivanavicius et al., 2007) found that MIA induces synovitis in the joints of rats 1-3 days post-injection, followed by gradual thinning of articular cartilage and subsequent lesions of subchondral bone from day 8–14 onwards. If MIA induces the same transient synovitis followed by structural changes in articular cartilage and bone in pigs, this may explain the biphasic decrease in weight-bearing in pigs as well.

Interestingly, in animals injected with saline solution, increased asymmetry was observed on day 1 as well. The injection of fluids in the joint space may distend the joint capsule or cause a mild synovitis, resulting in pain. This effect has been observed in healthy human subjects for up to 24 hours after intra-articular injection (Jayson and St Dixon, 1970). In horses however, intra-articular injection of saline solution resulted in lameness that only lasted for 2 hours (Thomsen et al., 2010).

Cut-off values for CLT in this study were set at <-25 and >25. This yielded some positive animals in baseline measurements, although these animals were not visually lame. Since no diagnostic procedures were performed at that moment, it is impossible to know if these animals were really experiencing a lameness-causing condition or if they were false-positives. A previous study comparing clinically sound and lame animals did show clear cut-off points to distinguish between lame and sound animals (Meijer et al., 2014), however, this was a small-scale study in animals that were visually clearly experiencing lameness. More appropriate cut-off values may be derived from larger scale studies. However, since there is a large variation in CLT even in healthy animals which partly

overlap CLFs of sound animals, it may be difficult to establish a cut-offs value that does not yield false positive or false negative judgment. In addition, calculations of CLTs in this study were based on relatively low numbers of footfalls. It is possible that CLTs based on more footfalls yield better distinctions.

Observer scores showed correlations with all pressure mat CLTs. This finding is in contrast with two studies in dogs. In these studies, numerical rating scales did not correlate with force-plate derived variables, agreement between observers was low, and each observer seemed to apply an individual, unique scoring scale (Quinn et al., 2007; Waxman et al., 2008).

Visual scoring was performed under good conditions: pigs were alone in a large pen and were shown from all angles at both walk and trot. Observers could watch movies as often as they wanted. Both observers were experienced veterinarians and had received instructions prior to scoring the movies. In spite of these advantages, they reached only fair agreement, with observer B scoring consistently lower that observer A. However, they did not practice and compare scores beforehand, which may explain the lack of agreement.

Although significant joint damage was present in animals at necropsy, lameness (defined as a score of >1) was not observed in many MIA-injected animals. Although left-limb lameness defined as pressure mat CLT of <-25 were observed more often, it was still not present in all MIA-injected animals. There are several possible explanations for this discrepancy between structural joint pathology and functional impairment.

Osteoarthritis in weight bearing limbs is generally associated with altered range of motion of the affected joint (Steultjens et al., 2000), an (initial) altered spontaneous locomotor pattern (i.e. clinical lameness) and spontaneous and provoked pain (Creamer et al., 2000). However, the interrelationship between pathology and resultant pain, at least in humans, is variable. Although inflammation and joint damage are generally considered to contribute to joint pain, radiographic grading of the severity of osteoarthritis in humans often does not match the amount of pain reported (Dieppe and Lohmander, 2005).

Pigs, like many prey animals, tend to hide signs of weakness such as lameness, as it would leave them vulnerable to predation (Weary et al., 2008). Further, animals were trained to expect a sweet treat at the other end of the pen or runway. This positive anticipation, next to a possible direct effect of ingested sweet treats, which in children may or may not alter the pain experience (Harrison et al., 2015) may have competed with the expression of pain behaviour. It is therefore possible that pigs were not expressing lameness clearly

enough to be identified. This does, however, not mean that the pigs could not have experienced pain.

Cut-offs for pressure mat analysis were based on previous research in naturally-occurring lameness in pigs (Meijer et al., 2014). In that study animals that were visually lame (lameness scores of >2) were used, which means that cut-offs to identify more subtle forms of lameness may have to be set lower. Another possibility is that not weight bearing, but other aspects of gait, such as temporospatial characteristics, are altered in the MIA model of lameness, aspects that were not assessed by our kinetic pressure mat analysis.

The visual scoring system was modified, and several criteria that were present in the original scoring system by Main et al (Main et al., 2000) were not used in this study (table 2). These were mainly behavioural criteria (Main et al., 2000). It is possible that, especially because some aspects of lameness such as head bobbing are difficult to observe in pigs (Main et al., 2000), the usability and sensitivity of visual scoring is greatly enhanced by the addition of behavioural criteria. Disuse of these criteria for scoring lameness may have resulted in the reduced performance of visual scoring. Also, it is possible that scoring from video recordings is more difficult than expected, however, movie clips have been used to score lameness in several other studies (Fuller et al., 2006; Hewetson et al., 2006; Kaler et al., 2009; Keegan et al., 1998). Observers commented on some of the recordings as "difficult to score", which may mean that some of the errors are due to having to score from movies instead of live. On the other hand, in veterinary practice conditions are usually much worse, with low lighting intensities and crowded stables. The identification of lame animals by visual inspection is likely even more difficult under practice conditions, which means that many lame animals are probably never identified (false negatives).

Although MIA produces lesions that histologically and functionally resemble those of naturally-occurring osteoarthritis, there are some differences. The pathophysiological mechanism that causes these changes is not the same as in naturally-occurring disease. The comparison of transcription profiles from osteoarthritis cartilage of naturally-occurring osteoarthritis from humans and MIA- induced osteoarthritis rat cartilage showed that in less than 4% of genes from the rat cartilage that had altered expression profiles, this alteration was in the same direction as the human cartilage (i.e. both up-regulated or both downregulated) (Barve et al., 2007). So even though phenotype of naturally-occurring osteoarthritis in humans and MIA-induced osteoarthritis in rats may overlap, the biological processes at the base of the disease process are probably completely different.

Meloxicam treatment did not result in improved mean lameness scores or lower mean CLF's, except on day 56. At that timepoint all CLF except CLF of PVP had returned to baseline levels in the NSAID- treated animals, but in OA animals that did not receive NSAIDs CLF of PVF, LR and VI were still significantly lower than baseline levels. There has been much debate on the role of anti-inflammatory properties of NSAIDs on the progression of osteoarthritis (Ding, 2002). Meloxicam increased the *in vitro* synthesis of proteoglycans and hyaluronan in cartilage explants from patients with osteoarthritis and may therefore have a favourable effect on cartilage metabolism (Blot et al., 2000). *In vivo* studies on LPS-induced synovitis in horses showed a decrease of inflammatory markers in the synovial fluid in the first 24 hours following induction (de GRAUW et al., 2009). It is not clear, however, what the long-term effects of meloxicam on structural cartilage damage are.

The lack of effect of NSAIDs on lameness on other timepoints is in contrast to other studies on NSAID use in lame pigs (Friton et al., 2002; Pairis-Garcia et al., 2015). There may be several reasons for the discrepancy between the findings in the current study and Pairis-Garcia et al (Pairis-Garcia et al., 2015) chemically induced synovitis in sows and found that both meloxicam and flunixin meglumine increased maximum pressure put on a lame limb. This finding supports the notion that these compounds had pain-mitigating effects. This contrasts with our findings. However, it is possible that the inflammatory component of synovitis is more sensitive to the anti-inflammatory effects of NSAIDs than the early apoptosis and necrosis that is seen in the MIA model. Pain from severe osteoarthritis is notoriously difficult to treat and may be localized on several levels of the pain-processing pathway(Sofat et al., 2011; Zhang et al., 2013). For example, up to 15% of people that underwent total knee replacement still report persistent severe pain (Wylde et al., 2011). This suggests that in osteoarthritis, processes other than inflammation and joint damage, such as plastic neural changes resulting in peripheral and central sensitization (Arendt-Nielsen et al., 2015, 2010), also play a role in the resultant pain intensity and persistence of pain. Studies using high doses of MIA in rats have shown that, especially in later phases of the disease process, NSAIDs are not able to resolve hind limb weight-bearing asymmetry (Ivanavicius et al., 2007; Pomonis et al., 2005). In contrast, drugs used to treat neuropathic pain such as gabapentin, pregabalin, milnacipran and amitriptyline did result in decreased weight-bearing asymmetry (Burnham and Dickenson, 2013; Ivanavicius et al., 2007; Thakur et al., 2012). This suggests neuropathic pain component in animals with severe MIA-induced osteoarthritis and could explain the absence of an effect of NSAIDs.

A study in cats that assessed the effect of meloxicam, amongst others, on PVF using a pressure mat, also failed to find therapeutic efficacy. It was hypothesised that this may be due to the beneficial effect of repeated exercise that the pressure mat measurements provided (Guillot et al., 2013). Moderate exercise is regularly recommended as an

intervention that improves clinical outcome in human osteoarthritis patients (Bennell and Hinman, 2005) and may directly influence cartilage composition (Roos and Dahlberg, 2005). Although the pigs in our study exercised regularly at the beginning of the study, towards the end there were less testing sessions. It seems unlikely that this exercise regime ameliorated the symptoms of the severe osteoarthritis in our pigs in such a way that the effect of the NSAID was masked.

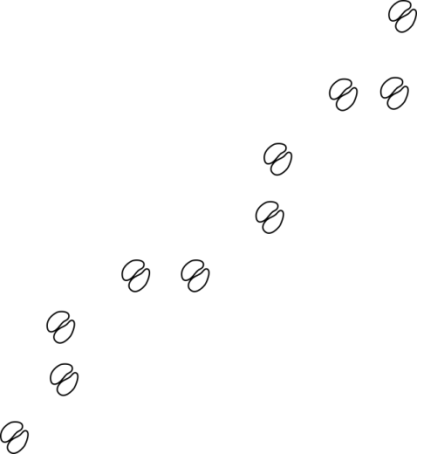
A last explanation for the absence of an effect of NSAIDs in this study is that the method we used is not suitable to detect its effects. Pressure mat analysis quantifies lameness, which is largely the functional result of the affective-motivational aspect of pain. It does not, however, quantify the cognitive-evaluative aspect of pain. Methods that measure this aspect, such as conditioned place aversion/place escape paradigms may provide more information on the pain lame pigs experience, and on the efficacy of NSAIDs in mitigating these effects.

Conclusions

The intra-articular injection of MIA induced both functional and structural changes in weaned piglets. Functional changes were characterized by decreased symmetry of gait as quantified by pressure-mat derived variables. To a lesser degree, functional changes were also identified by visual scoring of gait. Structural changes in cartilage, subchondral bone, synovial membranes and bone marrow were seen at post-mortem examination of the MIA- injected joints. Both histological changes and the temporal pattern of development of functional changes resemble those seen in rat models of MIA-induced osteoarthritis.

Daily administration of the NSAID meloxicam did not result in functional improvement of gait in the MIA-treated animals. There are several explanations for this lack of treatment effect. It is possible that meloxicam did not address all mechanisms that cause pain in MIA-induced osteoarthritis and therefor did not alleviate pain strongly enough to be observable. Also, very small treatment effects are not clinically relevant. Another possibility is that the methods that were used in this study (visual scoring and pressure mat analysis) were not suitable to detect the analgesic effect of meloxicam.

Although the intra-articular injection of MIA appears to induce functional and structural changes in piglets, the methods we used in the present study do not fully describe all clinical features. Possibly, other methods, such as mechanical threshold tests or conditioned place aversion/place escape tests are able to contribute to the full clinical picture.



6

General Discussion

6.1 Summary of results

The aim of this thesis was to evaluate the use of a pressure mat as a new method to quantify lameness in weaned piglets. Although a pressure mat has already been used in sows (Karriker et al., 2013; Pairis-Garcia et al., 2015), no normative data on pressure mat parameters of weaned piglets were available to date. Therefore, in **Chapter 2** the gait of piglets from 5 to 15 weeks of age was analysed weekly using a pressure mat. We assessed the effects of time (i.c. longitudinal measurement session), limb and velocity on four kinetic pressure mat parameters: peak vertical force (PVF), load rate (LR), vertical impulse (VI) and peak vertical pressure (PVP) in sound piglets. Velocity, measurement session and limb (fore- or hindlimb) all influenced pressure mat measurements, except for VI, which was not affected by velocity. It is hardly possible to control for these varying influences on runs on the pressure mat. This may hamper the detection of lame pigs, since the noise in the data due to these influences may be larger than the variation due to lameness. Therefore, to minimise the influence of velocity, measurement session and limb, a method to compare limbs to each other *within* each run was assessed. The asymmetry index (ASI) comparing contralateral limb pairs (left front vs. right front and left hind vs. right hind) provides a dimensionless ratio that quantifies the degree of asymmetry in loading of the limbs, including the direction of this asymmetry. ASI values can range from -200 to 200, in which -200 indicates that the left limb is not loaded at all, 200 indicates that the right limb is not loaded at all and zero indicates perfect symmetry. In dogs and horses asymmetry is considered a hallmark feature of lameness (Fanchon and Grandjean, 2007; Lequang et al., 2010; Oosterlinck et al., 2011), and ASIs were therefore adopted as a promising method to evaluate gait in pigs and quantify lameness. The amount of asymmetry observed in sound piglets was larger than that found in dogs (Oosterlinck et al., 2011) or horses (Oosterlinck et al., 2010a). ASIs of the piglets did not change over time.

In **Chapter 3** the use of ASIs of several pressure mat parameters to distinguish between clinically lame and sound pigs was assessed. Additionally, redistribution patterns in lame pigs were described. Ten clinically lame pigs and 10 sound controls were compared. The lame animals had significantly higher left-right ASIs than their sound counterparts. The ASIs correlated well with visual lameness scores, but the advantage of ASIs over visual scoring is that they are not depending on subjective observations. ROC curves yielded clear cut-off points to distinguish lame from sound pigs for all four pressure mat parameters.

Like lame dogs and horses (Fanchon and Grandjean, 2007; Oosterlinck et al., 2011; Weishaupt, 2008; Weishaupt et al., 2006, 2004), pigs tended to shift their weight away from the affected limb. ASIs comparing the lame limb to its contralateral, ipsilateral and

diagonal counterpart were all significantly higher in lame animals, indicating that weight is shifted towards the sound limbs. In some pigs this weight shifting was so profound that even ASIs comparing two unaffected limbs of lame pigs were significantly higher than zero (zero indicating perfect symmetry).

In **Chapter 4,** the sensitivity of ASIs to detect the effect of the analgesic buprenorphine was evaluated. Since pain is the most important reason for an animal to display lameness, it was hypothesized that a strong analgesic would -at least partially- restore left–right symmetry. This positive treatment effect was indeed observed for ASI of LR, VI and PVP. Although the level of locomotor asymmetry decreased with roughly 25-50% after treatment with buprenorphine, it did not return to values considered normal for sound animals. Furthermore, activity in an open field test increased when lame pigs were administered buprenorphine, indicating that activity may also be an interesting method to assess lameness. Buprenorphine did not affect open field activity in sound pigs.

We concluded from the results obtained in chapter 4 that the pressure mat was able to identify and quantify lameness, and that it was able to detect the treatment effects of a strong analgesic. Therefore, the pressure mat method was used as part of a multilevel pain assessment system to characterize the functional aspects of a lameness model in pigs in **Chapter 5**. The pig with an experimentally induced osteoarthritis was used as model in this experiment. MIA (monosodium-iodoacetate), an inhibitor of glyceraldehye-3-phosphate dehydrogenase activity in chondrocytes, was injected into the left intercarpal joint of 20 weaned piglets. Intra-articular injection of MIA resulted in increased asymmetry that lasted at least 28 days, which is comparable to the effect of MIA in rats (Fernihough et al., 2004; Pomonis et al., 2005). Oral administration of the NSAID meloxicam did not result in a decrease in asymmetry. Although overall asymmetry in the MIA-injected group was pronounced, ASIs of individual animals did not always reflect the pathology within the joint. From visual scores, however, it was even harder to detect and score lameness, indicating that the lameness induced by MIA has probably been very subtle in some pigs. The pig with MIA-induced lameness therefore is likely not the animal model of choice to assess pain caused by osteoarthritis and its treatment.

Pressure mats do not only provide kinetic data, but also provide temporospatial information. In **Appendix I**, ASIs of stance duration, step duration, step length and stance percentage of lame and sound pigs were compared. ASIs of all four parameters were significantly higher in lame pigs. Intra-class correlations, however, were poor, and the ASI of step duration was influenced by velocity. Quantifying lameness, purely based on ASIs of temporospatial parameters may not be advisable. However, they may be useful since they assess aspects of gait different from kinetic parameters. ASIs of temporospatial parameters may complement the objectively recorded clinical picture.

Pressure mat analysis can be used in a wide range of species. In **Appendix II**, normative data on both left-right and front-hind ASIs of healthy dogs were collected and custom made software to automatically assess these quadrupedal locomotor data was used. Side of guidance, handler, gait, gender and weight category influenced several parameters, which means that these factors need to be controlled for when designing experiments using pressure mat analysis.

Appendix III is an attempt to automatically quantify locomotor activity in weaned pigs using accelerometers. Although it was practically possible to collect data on activity in group-housed animals, no difference in activity between lame and sound pigs was found using activity parameters as collected with accelerometers.

6.2 Lameness detection and quantification in pigs

6.2.1 Visual scoring

Visual scoring is still the clinical tool of farmers and veterinarians for detecting lameness in pigs, and this technique is often used in welfare assessment protocols. It is also frequently applied in research settings (Etterlin et al., 2015; Munsterhjelm et al., 2015; Quinn et al., 2015), but the low sensitivity of visual scoring, in particular in detecting animals with mild and moderate lameness, is considered an important reason for unexpected results (Quinn et al., 2015).

Notwithstanding their drawbacks, there are several reasons to use visual scoring systems. They do not require costly or elaborate setups and are to this day the only feasible method to quantify lameness in a group of pigs at farm level. Throughout this thesis, visual scoring was used next to pressure mat analysis for comparison reasons.

Either the original system developed and described by Main et al (Main et al., 2000) or a modified version of it was used. This system consists of a numerical rating scale with categories ranging from 0 (no abnormality in posture, gait, and/or behaviour) to 5 (severely lame pig, incapable of standing). There were several reasons to choose this system. First, it was designed especially for weaned and finishing pigs. Secondly, it provides descriptors of gait, but also descriptors of behaviour. Thirdly, its repeatability and usability by either trained or untrained observers has been documented (Main et al., 2000).

In Chapter 4 and 5, subtle changes in lameness were assessed. Visual scoring did not perform well in the detection of these subtle effects. The effect of buprenorphine on lameness, although clearly identifiable on both the pressure mat analysis and the activity levels of the pigs, was not noted using visual scoring. Early-stage osteoarthritis caused by

MIA injection was hardly detected by visual inspection. Mean gait scores on day 1 and 28 after MIA injection were slightly above 1 ("abnormal stride length, movements no longer fluent") but never reached score 2 ("lameness detected, shortened stride"). Some individuals did present with a gait score of 2 or higher, but in most of these cases the lameness was not observed on all time points or was only identified by one of the two observers. It is possible that the 6-point categorical scale did not provide enough detail to score small changes in lameness. Visual analogue scales have a much larger range of available scores and may therefore be more suited to record small differences, but according to some authors this comes at the cost of reduced agreement between observers (Engel et al., 2003; Quinn et al., 2007). "Tagged" visual analogue scales, labelled with clear descriptors, may provide a useful tool as they retain the possibility of indicating small differences, but with inter- and intra-observer agreements that are comparable to, or even exceed ordinal scales (Nalon et al., 2014).

In this study, visual scoring was performed under nearly "ideal" conditions (bright lighting, pig filmed from all angles while walking and trotting in a pen without other pigs). A fair amount of time was spent grading each pig (movies were approximately one minute long, and were sometimes watched twice by observers). In practice, conditions are usually much worse and the available time to observe individual pigs is limited. It is conceivable that t many (subtle) lameness cases remain undetected. Lameness detection in practical settings therefore remains challenging.

6.2.2 Pressure mat

Gait adaptations in lameness

Lameness can be described and observed in several ways. In visual scoring systems, "the clinical eye" mostly evaluates symptoms such as weight redistribution, step length, head bobbing, arched back, fore limbs or hind limbs turned out and stiffness (Grégoire et al., 2013; Kirk et al., 2005; Main et al., 2000; Nalon et al., 2013a). In horses, lameness is often divided into stance-and swing phase lameness, although this remains a point of discussion (Back and Clayton, 2013b). Pressure mat analysis is able to measure several aspects of lameness. In this thesis, the focus has been on weight redistribution.

If an animal experiences pain when it puts weight on a limb, it will attempt to minimise this pain by *reducing load* in the lame limb (resulting in lower Load Rate and Peak Vertical Force) and *redistributing* the load to other limbs (Weishaupt, 2008). *Load reduction* in the lame limb can be achieved by two major mechanisms: smoother loading of the lame limb and decreasing the vertical loading by adapting the movements of head and trunk (Back and Clayton, 2013b). Although no absolute values for Load Rate were presented in Chapter 3, it was shown that Load Rate ASI (comparing the Load Rate of the

lame limb to that of the sound limbs) was significantly higher in lame pigs. The other load reducing mechanism, decreasing the vertical movement of head and trunk, could not be measured by the pressure mat, since it only collects data on claw-mat interaction. It has been suggested that the relatively short neck of pigs limits the vertical movements of the head and that this may be one of the reasons that the identification of lameness in this species is difficult (Main et al., 2000). However, a recent study measuring vertical head displacement using kinematics (S. Stavrakakis et al., 2015) reported a higher displacement in lame pigs.

Load redistribution has been shown in several species, for example horses (Maliye et al., 2015; Weishaupt, 2008), dogs (Abdelhadi et al., 2012; Bockstahler et al., 2009; Fischer et al., 2013; Rumph et al., 1995) and cattle (Scott, 1989). Generally, most of the load is shifted from the lame limb towards the contralateral limb, and to a lesser extent towards the ipsilateral and diagonal limb. Previous work has shown that lame sows shift their weight towards the contralateral limb (Karriker et al., 2013) and also towards the other limbs (Johnson et al., 2011). Comparable redistribution patterns were found in weaned piglets in Chapter 3. Thus, it can be concluded that pigs use similar strategies to decrease load put on an affected limb by mechanisms that are comparable to those used by other species.

Although in the main part of this thesis only kinetic parameters were assessed, in Appendix I the temporospatial parameters stance duration, step duration, step length and stance percentage have also been assessed. Although the mean ASIs of these parameters in lame pigs were significantly different from zero (indicating perfect locomotor symmetry), intra-class correlations were poor, indicating large variability within these ASIs. This experiment showed that temporospatial gait characteristics in lame pigs are adapted in a way that is comparable to that of other species (Buchner et al., 1995; Flower et al., 2005; Keegan et al., 2000; Weishaupt et al., 2006).

Practical considerations

Whether or not a method is deemed to be practical partly depends on the circumstances under which it is applied. Important considerations when assessing the usability of pressure mat gait analysis are: training needed, time investment, and cost. The installation and use of a pressure mat system itself are fairly straightforward and require no prior training.

The time invested in data acquisition, -processing and –analysis however is a major concern for routine, high-throughput pressure mat analysis when the Footscan system is used. Data acquisition can be greatly simplified by providing a suitable corridor that promotes walking in a straight line, reduces distraction during the run, and prevents pigs

from leaning against the wall. This minimizes the amount of runs needed to obtain enough valid data. Training itself however is time-consuming and requires a lot of manpower, so it is only feasible in research settings when measures are taken from the same pigs at several time points. Data processing and –analysis is also a very time-consuming process. Since the Footscan software is designed for use in human subjects, footfalls from pigs need to be identified and assigned to the correct foot manually. Identification of the footfalls means that either the software or the human observer needs to "tell" the program which pixels/sensors belong to a particular footfall and which ones are either "empty" or belong to another footfall. This seems fairly straightforward, but in the case of pigs (and especially in larger pigs that have sloping pasterns) sometimes the dewclaws touch the pressure mat and make a small pressure point independently from the main footfall. Even though this pressure point does not contribute much to the entire pressure profile of a foot, they may be mistaken for a separate footfall if rolloff patterns and in some cases video material are not carefully examined. The free "Pawlabeling" program developed by Flipse RD (Flipse, 2013) is able to perform this step in data processing faster than a human observer, and provides basic visualisations of results. It may therefore be a valuable aid in the use of Footscan pressure mat analysis for research purposes. The use of the "Pawlabeling" program requires some basic knowledge on the installation of Python-based programs, but is otherwise fairly straightforward.

Cost may be another important consideration for the use of pressure mat analysis. Prices depend on the size of the mat and the software options that are provided. Scientific systems like the one used for this thesis cost around € 20.000 and may therefore be considered too expensive for use in practical (farm) setting. Cheaper options (smaller mats and/or simpler software) are available and start around € 4500, which is still a considerable investment. However, prices for technology generally seem to decline so in the future these systems may become more affordable.

Theoretical considerations

Pressure mats can only measure interactions between the claw and the floor/mat. This has two possible consequences: first, some forms of lameness may be missed because they are expressed in a way that cannot be measured by the pressure mat (for example, when joint angle patterns are altered), and secondly it is generally not possible to localise the site of the lameness-inducing lesion within the limb.

Influencing factors Several factors may influence pressure mat parameters. In pigs, like in horses [3, 4] and dogs [5–9], velocity had an important effect on most pressure mat parameters. The exception was VI, in which no effect of velocity was seen. In kinetic studies on horses and dogs, animals are often led across the pressure mat or force plate. This makes it possible to control the velocity and keep it within a relatively narrow range.

In pigs, this is very difficult and therefor the influence of velocity on pressure mat parameters is an important obstacle.

In addition to the effect of velocity, there was also a significant effect of measurement session. In our young, growing pigs the measurement sessions were 1 week apart, which means that there may have been several factors contributing to this effect, for example differences in conformation in the pigs, but also small differences in environmental factors. In dogs, measurement session is known to be the second most important source of variation in force plate data, after velocity [10]. In our pigs, the effect of growth and changing body conformation may have been an additional source of variation. It is known from horses (Back et al., 2007b) and dogs (Mölsä et al., 2010; Voss et al., 2011) that different conformations may lead to differences in gait. Lastly, there was a significant effect of limb (fore- or hindlimb) on all pressure mat parameters. Like many other quadrupeds [7, 11–13], pigs appear to carry more load on their front limbs than on their hind limbs (Thorup et al., 2007; Von Wachenfelt et al., 2008).

Asymmetry indices ASIs were used as a process to overcome some of these obstacles. Symmetry is considered an important feature of normal locomotion, which is already implied by the naming of so-called "symmetrical gaits", such as walk and trot.

The advantage of ASIs derived from pressure mat measurements is that limbs from an animal and within one run are compared to each other. This minimises the effect of inter-run variations that may be caused by, for example, velocity differences. In pigs from 5 to 15 weeks old, age did not influence ASIs, in contrast to absolute pressure mat parameters in which age did have an influence. This makes ASIs particularly suitable for experiments that follow animals over time.

Perfect symmetry (an ASI of zero) was used throughout this thesis as indicative of a sound animal. The assumption that a sound animal moves symmetrically, however, has been challenged. In humans, symmetry indices of up to 7.6 for PVF were found in clinically healthy subjects (Herzog et al., 1989). Limb dominance, related to functional differentiation of the two brain hemispheres, and functional differentiation between limbs have been proposed as reasons for the frequently-observed gait asymmetry in healthy humans (Sadeghi et al., 2000). Limb dominance has been shown in dogs (Colborne et al., 2011, 2008) and horses (M. Oosterlinck et al., 2011) and may also be present in pigs. Although in Chapter 2, 3 and the supplemental materials of Chapter 4 mean ASIs of sound pigs approached 0, it was extremely rare to find actual ASIs of 0 in individual animals. Furthermore, although this was not analysed, it seemed that some sound animals had a tendency to favour either the left-or the right limbs. This would suggest that a) some asymmetry is present in healthy animals as well and b) that this asymmetry is, at least in some animals, more inclined towards one side.

Although asymmetry indices were not influenced by velocity, measurement session or limb, there may be other factors that influence them. In Appendix II, it was shown that gender, gait (walk or trot), familiarity of the handler and side of the handler may influence left-right and front-hind ASIs of clinically sound dogs. Handler effects were not present in our pigs, since they crossed the pressure mat voluntarily and independently. From visual assessment of the data, gender was not expected to influence kinetic parameters and it was therefore not assessed in the model used in Chapter 3. In appendix I, no effect of gender on temporospatial ASIs in pigs was found. However, in Appendix II, gender influenced ASI of VI but not stance duration in dogs.

In conclusion, although ASIs are less likely to be influenced by external factors than absolute variables, there may be some unknown influences that may affect ASIs as well.

Replicability and sensitivity/specificity Although replicability within measurement session was fair to excellent for the kinetic pressure mat parameters, in some instances considerable variation in the data of individual animals was seen. Especially in lame animals, ASIs sometimes varied widely. In humans, larger within-subject variability in measurements of individuals with pathology is a well-known phenomenon (Hausdorff, 2005; Hausdorff et al., 1998; Steinwender et al., 2000).

Variability in gait data may be either due to internal variability such as natural fluctuations, pathologies, the effect of aging, or external variability such as environmental factors, random variation and methodological errors (Chau et al., 2005). Sources of internal variability within the pigs may be the previously mentioned effect of the presence of pathology, but also natural variability in gait. This natural variability has been studied in human subjects (Hausdorff et al., 1996, 1995), but virtually no information on this effect in pigs is available. External variability was minimised as much as possible. The same pressure mat was used throughout the experiment. One experimenter performed all pressure mat measurements. Selection and assigning of footfalls was not always done by the same person in the studies of this thesis, but within one experiment always the same person selected and assigned the footfalls. Pressure mat analysis was, however, not always performed on the same time during the day. Also, only a relatively small amount of runs per pig per time point was collected. Collection of more runs and thus footfalls per pig may be advisable, but this is neither always feasible or nor is it ethically acceptable in animals that are severely lame.

A consequence of larger variability is decreased specificity. Since there is large variability, in particular in lame animals, sometimes ASIs of lame and sound animals overlap. From gait samples that are highly variable large effects are easier to detect than more subtle changes (Chau et al., 2005). Lame animals in Chapter 3 were selected on-farm based on visual inspection of gait. As stated previously, this is challenging in itself. Therefore, it is

likely that only fairly severe cases of lameness were identified. Consequently, identifying lameness in these animals using a pressure mat was straightforward.

The effects of an analgesic, such as the μ-opioid antagonist buprenorphine or the NSAID meloxicam, are often not sufficiently strong for completely reversing lameness (Borer et al., 2003; Hazewinkel et al., 2008), and the differences between pre-treatment and post-treatment values are therefore usually more subtle than the difference between lame and sound animals(Tapper et al., 2013). Even so, in Chapter 4, using the pressure mat, we were able to identify the effects of buprenorphine in lame animals. We did not, however, observe effects of meloxicam on experimentally induced osteoarthritis (Chapter 5). This may have been due to insufficient sensitivity of the pressure mat to identify this effect. Based on both ASI and visual scores, the lameness in animals that received an intra-articular injection of MIA was much less severe than the naturally-occurring lameness in the animals used in Chapter 4. It is possible that more animals are needed to reach sufficient power to demonstrate an effect of the analgesic drug. In addition, meloxicam may not have a strong enough analgesic effect in these animals. It is know from human subjects that, although NSAIDs provide some pain relief, this pain relief is often not effective in severe cases, necessitating joint replacement (Dieppe and Lohmander, 2005; Smith et al., n.d.). Possibly the effect of NSAIDs in pigs in Chapter 5 was too small to quantify with pressure mat analysis and the effect may not be clinically relevant.

Measuring lameness and measuring pain

As stated previously, lameness is often caused by pain and attempts of an animal to minimise the pain by reducing the load put on the affected limb. Gait analysis is therefor sometimes cited as an indirect measure of pain (Cobos and Portillo-Salido, 2013; Gregory et al., 2013; Mogil, 2009; Mogil and Crager, 2004). Indeed, in Chapter 4, gait asymmetry decreased in response to administration of an analgesic, suggesting that what is measured is indeed (partly) due to pain. However, gait asymmetry is not a direct measure of the pain that an animal experiences and should not be interpreted as such, for several reasons.

First, although gait alterations are often caused by pain, this is not true for all cases. For example, mechanical restrictions may cause altered gait but not pain. Some neurological deficits are not painful, but do induce gait alterations, for example cortical lesions such as those induced by brain infarction or haemorrhage or by traumatic brain injury (Patterson et al., 2008). Secondly, particularly prey animals tend to hide signs of weakness because it would make them more susceptible to predation. Therefore, gait asymmetry is not directly translatable to an index of pain (Short, 1998). The fact that an animal is not showing any outward signs of pain does not mean it is not experiencing pain. Pain can be measured on several levels (Gregory et al., 2013; Mao, 2012; Mogil, 2009). The sensory-

discriminative component of pain provides information on the localization and intensity of the stimulus, the affective-motivational component enables an individual to (negatively) rate the experience and react accordingly, and the cognitive-evaluative component enables the individual to learn from the experience in order to avoid it in the future (Auvray et al., 2010; Melzack and Casey, 1968). Measuring asymmetry is a way to say something about the affective-motivational aspect of pain, but it does not address the cognitive-evaluative aspect of pain.

In summary, although lameness can be caused by pain and pressure mat analysis can quantify this effect of pain, caution should be exercised when interpreting ASIs as outcome parameters for pain experience of an animal.

6.2.3 Measuring activity

It is known from previous studies in cattle (Blackie et al., 2011; Chapinal et al., 2009; Walker et al., 2008) and sows (Grégoire et al., 2013) that lame animals tend to be less active. When lameness is caused by a painful process, the animal will try to avoid pain as much as possible by limiting locomotor behaviour and lying down. Additionally, when lameness is caused by inflammatory processes they may trigger a pro-inflammatory cytokine release which in turn induces sickness behaviour (including inactivity) (Kelley et al., 2003). Monitoring activity may therefore provide an additional method to detect lame animals.

Since pigs tend to spend a large proportion of the day lying down, visual activity monitoring can be time-consuming. In Chapter 4 we therefore tried to stimulate exploratory behaviour by placing animals in a novel environment, an open field arena, thereby shortening the time needed to observe differences in locomotor behaviour. Another approach was used in Appendix III, where we attempted to automatically monitor activity in the home pen using accelerometers.

The effect of an analgesic on activity in lame animals was clearly visible when scoring locomotor activity in the open field test. Animals became considerably more active when they were treated with buprenorphine. In contrast, sound pigs treated with buprenorphine did not alter their activity in the open field.

Activity monitoring using manual scoring of behaviour is time-consuming and labour-intensive. Therefore, a method to automatically assess activity, namely accelerometers, was investigated. Automated activity monitoring using accelerometers has already been used to quantify locomotor behaviour in sows, the effect of lameness upon activity, and the effect of NSAIDs on activity in lame sows (Conte et al., 2014a; Cornou and Lundbye-Christensen, 2008; Escalante et al., 2013; Grégoire et al., 2013b; Ringgenberg et al., 2010).

In weaned piglets, however, there was no difference between activity patterns of lame and sound animals, as measured by accelerometers, found (Appendix III). This contrasts with the effects of lameness on open field behaviour seen in Chapter 4. However, activity in the open field test is stimulated by novelty, whereas activity using accelerometers was assessed under normal housing conditions of the pig. Therefore, it is possible that there really was no difference between lame and sound pigs under normal housing conditions. Another possibility is that the method (accelerometers) is not suitable to detect the effect of lameness on activity.

Lameness and welfare

In Chapter 1 the following definition of welfare was cited: an animal is in a positive state of welfare when it is able to "*adequately react to hunger, thirst, thermal or physical discomfort, injuries or diseases, fear and chronic stress, and thus has the freedom to display normal behavioural patterns that allow the animal to adapt to the demands of the prevailing environmental circumstances and enable it to reach a state that it perceives as positive*" (Ohl and Van Der Staay, 2012). In this definition, the ability to react appropriately, i.e. to adapt, to both positive and negative stimuli is central.

Lameness is often an adaptation of the animal to a painful process. This was confirmed by the findings presented in Chapter 4. In that experiment, the administration of an analgesic resulted in decreased locomotor asymmetry, suggesting that lameness, quantified as locomotor asymmetry was (at least partly) caused by experiencing pain.

Adapting gait by shifting weight bearing from the affected limb is an adaptation strategy to reduce pain. In the short term, this may be effective in diminishing pain and may promote healing. On the long run, however, these compensatory movements may put excess strain on the other limbs and predispose the animal to secondary injuries (Meershoek et al., 2002; Weishaupt et al., 2006).

Apart from shifting weight away from the affected limb, lame animals may also be less active. In Chapter 4, lame animals that were administered an analgesic became more active in an open field test. It may be argued that decreased locomotor activity is a behavioural strategy of the animal to reduce pain, i.e. that by adapting its behaviour the animal is able to adequately react to the lameness-causing injury. As already outlined in Chapter 1, locomotor behaviour in itself does serve several important functions such as reaching food and water. A secondary effect of reducing locomotor behaviour may mean these functions cannot be performed adequately anymore. This may lead to hunger and thirst, since the animal does not visit feeding and drinking stations as often as it would do when it were healthy (Cornou et al., 2008; Madec et al., 1986). Also, a lame pig may be

unable to avoid unwanted social interaction with penmates, which in turn can lead to stress and injuries (Heinonen et al., 2013).

Pigs that are lame also lie down more. Although this was not confirmed in the experiments described in this thesis, this adaptive behaviour, providing relief from pain, has been found in sows (Grégoire et al., 2013). It is important that the animal has a comfortable surface to rest on and that is not harassed by penmates. In current group-housing systems for weaned piglets, flooring is usually hard (either concrete or plastic) and there is little possibility to escape and/or hide. As a consequence, lying down will probably not result in sufficient improvement in welfare for most pigs.

In order to minimize the decreased welfare due to pain in lameness, both the duration and the intensity of the pain can be modified. In cattle, duration between the onset of lameness and the start of treatment can be considerable and in individual cases it may take up to 7 weeks before treatment is initiated (Alawneh et al., 2012). No data on intervals between detection and treatment of lameness in pigs are available, but since lameness detection by visual inspection is problematic under farm conditions it is likely that similar delays are present in the initiation of treatment of lame pigs. Early diagnosis of lameness in cattle was associated with lower lameness recurrence rates 4 weeks after initial treatment (Leach et al., 2012). Furthermore, long-lasting pain may result in central sensitisation and development of a chronic pain state that is difficult to treat (Muir III and Woolf, 2001; Woolf and Chong, 1993). The intensity of the pain experience may be modified by the use of analgesics. It is widely accepted in veterinary medicine that a multimodal approach to analgesia, in which several drugs or techniques are used that target different levels along the pain pathway, is more effective and safer than targeting only one aspect along the pain pathway (Lamont, 2008). Unfortunately, only a limited number of analgesic compounds are registered for use in lame pigs. This limits the possibilities for multimodal approaches to analgesia and may mean that many painful conditions cannot be treated adequately, as was shown from the lack of effect of an NSAID on lameness caused by experimentally induced osteoarthritis in Chapter 5. Additionally, individual differences in efficacy of analgesic compounds between human subjects is well known (Moore et al., 2010), and very likely also exists between animals (Mogil, 1999; Wilson et al., 2002). Consequently, not every individual may experience adequate pain relief even when established therapeutic doses are used. It is therefore not only important to identify lame animals as early as possible, but also to initiate treatment soon and to monitor the effect of the treatment adequately. If treatment does not result in discernible improvement and to restoration of function, timely euthanasia may be the only available option to stop suffering of the animal. The development and registration of analgesic compounds that are both effective and safe for use in food-producing animals should be pursued.

Welfare of food-producing animals is an important concern for consumers of animal products (Eurobarometer, 2007). Quality labels indicating animal welfare may help consumers choose meat products that are in concordance with their preference for animal welfare standards (Blokhuis et al., 2003; Kehlbacher et al., 2012). Animal welfare assessment for these labels is performed using welfare assessment protocols. Since lameness is considered an important reason for impaired welfare in pigs (T. B. Jensen et al., 2012), it is used in several of these welfare assessment protocols as an animal-based welfare indicator. An example of such a protocol is the Welfare Quality®protocol, in which lameness is scored visually on a 3-point scale. Using welfare quality labels, more transparency on the welfare of food-producing animals will be achieved, helping promote market-driven welfare improvement and societal sustainability of meat production (Blokhuis et al., 2003; Heerwagen et al., 2014).

In conclusion, welfare is often impaired in lame animals. Although altered gait and decreased activity may be considered adaptive behaviours to minimize pain and promote healing, these behaviours often are either ineffective or even harmful to the animal. Pain management therefore is an important part of the treatment of lameness in pigs. Only a few options are, however, available and these may not be sufficiently effective in all individuals. There is a need for more analgesic compounds labelled for use in pigs and other farm animals. As consumer concerns over the welfare of food-producing animals grow, the adequate treatment of lameness in pigs may not only be relevant to the welfare of affected animals, but may also have positive economic implications for farmers, because products derived from animals kept under better welfare conditions can be marketed at a higher price.

6.3 Applications of pressure mat gait analysis

6.3.1 Research settings

The focus in this thesis was on pressure mat analysis as a tool to routinely quantify lameness in weaned piglets. The technique, however, is not limited to the use in this age group, or even this species. The pressure mat can withstand the weight of an adult draught horse, so maximum weight is not a limiting factor, whereas the mat size can be limiting. Since velocity cannot easily be controlled in pigs and is an important influencing factor for many gait parameters, it is important to calculate ASIs from data derived in one run. The width of the system we used (40 cm) may not be sufficient for large pigs. Pressure mat analysis has been successfully used in sows (Karriker et al., 2013) using a larger pressure mat system from a different manufacturer. The sensitivity of the pressure mat is high enough to assess the locomotion of small pigs, for example very young piglets or small breeds. The pressure profiles within a hoof of these animals however may not be

sufficiently reliable, since only a few sensors will be activated by very small claws. This will result in very low resolution pictures of the pressure profile. In Chapter 2, ASIs were shown to be consistent over time in young, growing pigs. This is interesting since it enables experimental setups in which animals are followed over time.

Pressure mat analysis may be used as an objective method to quantify the effects of interventions. In Chapter 5, a pharmacological intervention was assessed. The effect of other pharmacological interventions, such as the use of antibiotics, may be assessed as well. Of course interventions need not to be limited to pharmacological approaches. The relationship between flooring and gait and lameness has been the subject of previous research (Stavrakakis et al., 2014a; Thorup et al., 2008, 2007). The possibility to evaluate pressure profiles within a footprint, and thus to detect areas that are subject to high pressure, may provide interesting insights into the interaction between floor and claw (Carvalho et al., 2009; Van Der Tol et al., 2003), and the role of peak pressures in the development of claw lesions.

Gait defects are not only caused by orthopaedic disease. Neurologic deficits may also induce gait alterations (Rubino, 2002; Straw et al., 1999) and in humans are considered to be related to cognitive decline in elderly humans (Morris et al., 2016). In piglets, a neurological cause of altered gait is ataxia. Clinically, ataxia may arise from lesions in 3 locations: the vestibular system, the cerebellum and the spinal cord (sensory). Ataxic pigs can be very hard to identify and quantification of the amount of ataxia may be even more difficult than quantification of lameness. In humans, dogs and horses several conditions leading to ataxia have been quantified using pressure mat analysis. Gait alterations due to neurologic deficits can present in both kinetic (Ishihara et al., 2009) and temporospatial (Gordon-Evans et al., 2009; Patterson et al., 2008) parameters, which all can be measured by the pressure mat. Additional research will have to show whether it is possible to quantify ataxia using pressure mat analysis in piglets as well. If this is feasible, interventions to treat conditions that cause ataxia may be evaluated with pressure mat analysis in the same way as is used for detecting and quantifying lameness.

Pressure profiles under the claws are not the only area of interest. Pressure mats can also be used to measure pressure profiles under other parts of the body. An example is the study by Schubbert et al. (Schubbert et al., 2014), in which a pressure mat was used to assess the influence of rubber mats of different softness on peak force and contact area under specific areas of the body of gestating sows. Studying the effect of flooring on mean and peak pressure under body parts at risk for pressure sores (such as shoulders in sows) may yield information about optimal flooring for different ages of pigs, and may also be interesting for other species such as cattle.

Pressure mat analysis may not only be useful in veterinary research, but may also benefit research into human conditions. The use of pigs as experimental animals has increased over the years. They provide useful models of several human neurologic diseases (Lind et al., 2007; Swindle et al., 2012), for example stroke (Duberstein et al., 2014; Mangla et al., 2015) and traumatic brain injury (Jin et al., 2012). Gait analysis using a pressure mat might provide functional data on gait abnormalities in this kind of models.

In conclusion, pressure mat analysis can provide both kinetic and temporospatial data on gait, which makes it an interesting tool to study both orthopaedic and neurologic gait abnormalities. Furthermore, pressure distribution under other parts of the body may provide information on flooring that is comfortable for animals to lie on and that reduces the risk of injuries and pressure sores. Finally, this information may not only be useful for veterinary medicine, but can also provide functional information about gait abnormalities in certain animal models of human conditions.

6.3.2 Automated lameness detection on farm

As discussed previously, visual identification of lame pigs is challenging, even under favourable conditions. On farms, pigs are usually housed in large groups. This makes the detection of lameness even more difficult. Automated lameness detection would therefore be an interesting application for pressure mat analysis.

There are, however, several hurdles to overcome before this technique is usable under practice conditions. The first, and probably largest one is the computerisation of the data collection and analysis. The pressure mat system that we used is unable to assign footfalls of quadrupeds to the correct limb. There are, however, systems that can do that, such as the Tekscan pressure mats. The next step would then be to automatically verify if all requirements for a correct run are met. Only suitable runs and footfalls must be used, which requires the development of automatic filtering of runs and footfalls. A second obstacle is that there needs to be space in the stable that is suited for data collection. Feeding stations, as those that are used in group-housing for sows, may provide a feasible option, since sows are usually motivated to walk towards their food. It would be possible to construct a runway that leads to the food dispenser, which is long enough to incorporate a pressure mat. Unfortunately, feeding stations are not used in all sow stables, and are almost never used in stables for weaned and finishing pigs. The use of pressure mats for automated lameness detection will therefore not be implemented easily.

Some other gait analysis techniques have shown promising results; however these have only been used in sows until now. Static measurements of force stance variables in sows using four separate force platforms showed differences in weight distribution, number of

kicks and weight shifting between lame and sound sows (Pluym et al., 2013), but the system needs more validation before it can be used in practice. Another interesting option may be the use of relatively cheap motion sensors (i.e. the Microsoft Kinect® system) to detect kinematic characteristics of gait in pigs. Such a system has been used to assess, for example, head bobbing in sound pigs and may eventually be useful to detect lame pigs. For now however, tracking body segments still depends on markers that are placed on the pig. This is not feasible in practice, since it necessitates extensive handling of animals. Furthermore, trackers may be removed by other pigs in the pen. Reliable markerless tracking thus remains an important hurdle to overcome if this system is to be used in practice. In summary, although efforts to develop automated detection of lame pigs on farms are showing promising results, all these systems still need to be developed further before they can be considered as useful under practice conditions.

6.4 Conclusions

Pressure mat kinetics in sound pigs are influenced by several factors, of which velocity is the most important one. This poses a problem, since pigs are difficult to lead over a pressure mat and prefer to locomote at their own chosen velocity. A solution to this problem may be the use of ASIs. These indices compare footfalls from one animal within one run to each other, minimising the influence of between-run variability. Although we did not find factors influencing ASIs of kinetic parameters in pigs in our studies, ASI of the temporospatial parameter Step Duration was influenced by velocity. Step duration ASI may therefore be less suitable to use in pressure mat analysis of gait in pigs. , In dogs, pace, gender and handler familiarity and –side influenced some ASIs. This means that when designing experiments in dogs using pressure mats, these factors need to be controlled.

Asymmetry indices are able to objectively detect and quantify lameness in piglets in a way that correlates with visual scores. This is true for ASIs of both kinetic and temporospatial parameters. When lame animals are treated with the strong analgesic Buprenorphine, that effect can be detected by the pressure mat. This is in contrast with visual scoring, from which no improvement after analgesic administration could be detected. When the effect of a NSAID (meloxicam) on experimentally-induced lameness was assessed, no effect on visual lameness scores or on ASIs of kinetic parameters was found.

Next to quantifying lameness using gait parameters, the quantification of decreased locomotor activity may be used to detect lameness. Administration of a strong analgesic (buprenorphine) significantly improved activity of lame piglets in an open field test. Quantification of the activity of piglets within their home pen is time-consuming, since

automated measurements using accelerometers were unsuccessful in distinguishing lame from sound piglets and because every pig in the pen must be equipped with an accelerometer.

Pressure mat analysis of gait in pigs is a useful research tool to assess normal gait, to quantify lameness and the effect of therapeutic interventions. Further development, however, is needed before the pressure mat can be considered as a practical, high-throughput tool for detecting lame animals on farms and for characterising neurological gait abnormalities.

Appendix 1

Analysis of four spatiotemporal variables derived from pressure mat measurements: exploring their use for detecting and quantifying piglet lameness

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Under review

Abstract

Lameness is one of the main problems in modern pig industry. Apart from economic losses due to lower productivity and survivability, lameness severely impairs the animal's welfare. In pigs, lameness is often underdiagnosed due to the limited time spent observing individual animals and the absence of a fast, sensitive and appropriate diagnostic tool. Recent studies show promising results using different kinetic variables obtained by a pressure mat in detecting lameness. In the present study pressure mat analysis has provided data on the variables stance duration, step duration, step length and stance percentage. After training sound control (n=21) and lame piglets (n=9) to trot over the pressure mat, two valid measurements were registered and the data were analysed using the purpose-built program Pawlabeling. Average left/right asymmetry indices (ASI) were calculated for the fore- and hind limbs separately. The influence of velocity and lameness on these asymmetry indices was assessed using either a linear mixed model or a general linear model.

Significantly higher ASI means were found in both the affected and non-affected side (front/hind) of lame piglets compared to the controls for stance duration, step duration, step length and stance percentage.

Although these four pressure mat variables appear to provide promising indices for piglet lameness, the practical applicability remains to be determined, due to the difficulty to obtain enough valid claw strikes, the complexity of data analysis, and the lack of reference values.

Keywords: Kinetics, Gait analysis, Pig, Symmetry

Background

Following fertility disorders, lameness is one of the main problems in modern pig industry, accounting for 10% to 20% of all removals in sows (S. S. Anil et al., 2009; D'Allaire and Drolet, 2006). A cross-sectional study in the United Kingdom revealed an estimated prevalence of lameness of 14.4% in pregnant gilts, 16.9% in pregnant sows and 19.7% in finishing pigs (KilBride et al., 2009). Lameness in pigs is mostly associated with pathologies or injuries either in the foot itself, or the bones or the joints, caused by infectious arthritis, physical injuries, or osteochondrosis (D'Allaire and Drolet, 2006; T. B. Jensen et al., 2012).

Apart from the monetary losses due to high costs for treatment, lower productivity and lower life expectancies, lameness is a problem that severely impairs the animals' welfare (D'Allaire and Drolet, 2006). The animals might suffer from pain related to the cause of lameness and the accompanied reduced mobility. The problem is notoriously underdiagnosed in industrially kept animals because of the limited space in which pigs are housed and the shortage of time that farmers can spend to observe individual animals. Undiagnosed lameness can extend up to a level at which the animal is no longer able to stand up at all, consequently becomes dehydrated and undernourished, gets in danger of being overrun by its pen mates and of acquiring additional trauma. Because of the large negative impact of the problem on both animal and farmer, it is important to develop and validate a practical, fast and sensitive tool for diagnosing and treating lameness as early as possible.

Currently there are several possibilities to detect lameness, but they are either time consuming (kinematic analysis, clawprints in clay), or subjective to some degree (visual lameness scoring) (Grégoire et al., 2013). The latter method is the simplest technique in use. Main et al (Main et al., 2000) created a scoring system that incorporates gait characteristics as well as posture and behavioural aspects. This method has been shown to be highly replicable between trained observers, whereas the inter-rater reliability was poor between untrained observers. Consequently, this diagnostic tool is unsuited for occasional clinical investigations. Confirmatory studies in dogs and horses have also shown that visual methods are prone to subjectivity, mainly due to observer bias and lack of scoring experience (Arkell et al., 2006; Keegan, 2007; Main et al., 2000; Quinn et al., 2007a; Waxman et al., 2008). In particular subtle changes in locomotion occurring in early stages, such as weight bearing or posture, can easily be overlooked.

More objective methods such as kinematics and kinetics have widely been studied in horses and cattle. Kinematics has previously been used in pigs as well to study the effect of different floor surfaces on locomotion (Grégoire et al., 2013; Von Wachenfelt et al.,

2009), to quantify lameness in sows (Grégoire et al., 2013) and to discriminate between lame and sound pigs based on joint and stride kinetics (Stavrakakis et al., 2014b). This method is technically demanding, time consuming and is not applicable yet to practical situations.

Force plates and pressure mats can be used to collect kinetic data. However, force plates do not allow distinguishing between different feet when placed on the plate simultaneously. Proper data collection therefore demands either the use of multiple plates or collection of prints on a single plate in strictly controlled experimental conditions, and is therefore very time consuming. Footprint analysis using a pressure mat is not affected by these restrictions due to the dense array of pressure sensors with a high sampling frequency that enables discrimination between separate prints. It collects kinetic as well as spatiotemporal data of simultaneous and consecutive contacts. The knowledge about footprint analysis grows fast. It has been shown useful to evaluate gait patterns and pressure profiles in sound horses (M. Oosterlinck et al., 2011; Oosterlinck et al., 2010a), cows (Van Der Tol et al., 2003), dogs (LeQuang et al., 2010a), cats (Verdugo et al., 2013), sheep (Agostinho et al., 2012) and pigs (Meijer et al., 2014a). In addition, it has also been used to asses lameness in cows (Maertens et al., 2011), dogs (Lequang et al., 2010; Oosterlinck et al., 2011) and pigs (Meijer et al., 2014b). Most studies using pressure mats focus on kinetic data. The knowledge about alterations of parameters like stance duration, step duration, step length and stance percentage due to lameness is still limited.

In the present study we focussed on four locomotion parameters (stance duration, step duration, step length and stance percentage) of both healthy and lame three- to ten-week-old weaned piglets. The piglets trotted on a pressure mat which measured the data. We evaluated the asymmetry indices (ASI's) of these variables to determine whether this method was able to discriminate between sound and lame piglets based on observed differences in mean symmetry.

Results

Asymmetry indices

Average asymmetry indices are shown in Figure 1. Estimates for the magnitude of the differences are expressed as ratios. Estimated ratios with 95% confidence intervals for mean absolute ASI in affected and unaffected limbs of lame pigs are shown in table 1.

ASI Stance duration

The mean asymmetry indices for stance duration in lame piglets compared to those of the control piglets were significantly higher in the affected side (ratio=2.66, p=0.000) as well as in the non-affected side (ratio=1.71, p=0.020). In other words, the mean ASI of stance duration was 2.66 times higher in the affected side of lame pigs than in control pigs. The ASI of stance duration was not influenced by gender or velocity.

ASI Step duration

The mean asymmetry indices for step duration were higher in lame piglets' affected (ratio=3.01, p=0.000) and non-affected (ratio=1.74, p=0.029) side compared to those in the control group. There was a significant effect of velocity on the ASI for step duration ratio(ratio=2.33, p=0.001)

ASI Step length

The mean asymmetry indices for step length were higher in lame piglets than in control piglets, for both the affected (ratio=2.81, p<0.000) and the non-affected (ratio=2.65, p<0.000) side of lame piglets.

ASI Stance percentage

The mean ASI stance percentage were higher in lame piglets than in control piglets, both in the affected (3.50 times higher mean, p<0.000) and the non-affected (2.60 times higher mean, p<0.000) side of lame piglets (Figure 1, Table 1).

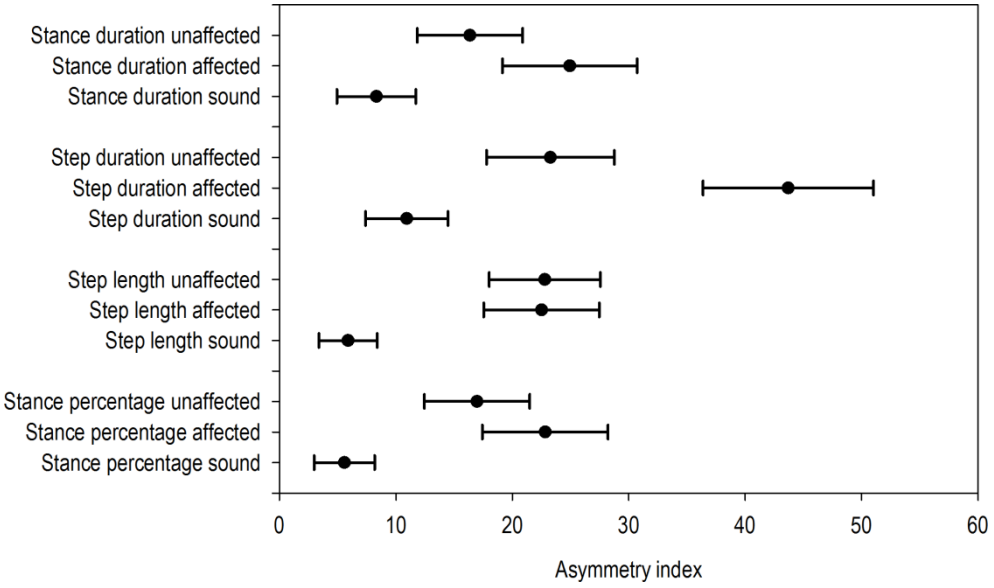

Figure 1. Overview of the non-transformed absolute mean asymmetry indices ± SEM in sound pigs and in lame pigs (both affected and unaffected side).

Table 1. Estimated ratios with 95% confidence intervals for mean absolute ASI between the affected and non-affected side of lame piglets compared to sound control piglets, shown for each pressure mat variable separately. All results are significant.

Variable		non-affected side lame piglets	affected side lame piglets
ASI stance duration	Estimate	1.71	2.66
	CI 95%	1.09 - 2.68	1.70 - 4.17
ASI step duration	Estimate	1.74	3.01
	CI 95%	1.06 - 2.85	1.83 - 4.93
ASI step length	Estimate	2.65	2.81
	CI 95%	1.63 - 4.33	1.72 - 4.57
ASI stance percentage	Estimate	2.60	3.50
	CI 95%	1.72 - 3.94	2.31 - 5.31

Intra-class correlations

Intra-class correlations (ICC) between asymmetry indices of piglets were generally poor (< 0.40), with the exception of the hind limb ASI of step length, which was fair (0.41) (Table 2).

Table 2. Intra-class correlation of ASIsof front- and hind limbs between runs per pig

	ICC front limb ASI	ICC hind limb ASI
Stance duration	0.080	0.109
Step duration	0.302	0.167
Step length	0.352	0.407
Stance percentage	0.077	0.296

Discussion

This study is the first to explore the use of the pressure mat variables stance duration, step duration, step length and stance percentage as a putative diagnostic tool for lameness in pigs. Collecting 5-7 initial runs per pig took on average 10 minutes per piglet, with no training needed. Data analysis however was a more time-consuming process, since the software used was designed for human gait analysis and thus unable to distinguish footprints of quadrupeds. The footprints had to be manually assigned left front, right front, left hind and right hind. Hereafter, the purpose build program 'Pawlabeling' [24] checked the measurements for discrepancies and calculated the ASIsautomatically per measurement. The use of "Pawlabeling" saved a considerable amount of time in the processing of the data, however, the manual assignment of footprints was time-consuming. The time needed for data analysis may not be of much importance for the use of pressure mat analysis as a research tool, but it is a major drawback for the routine use of the pressure mat system used in this study in clinical practice. There are pressure mats available that are able to automatically assign footprints and these may be considered for clinical use.

Although initially 3 runs per pig were collected, many of these runs did not meet the criterion of 8 valid contacts. This means that the entire run was discarded and none of the contacts was used. For some of the pigs this meant that they did not have the required 2 valid runs. Discriminating between valid and non-valid runs based on visual judgement was not possible during data collection. In addition, piglets with severe lameness were not forced to trot over the plate and therefor of these animals the runs often were of poorer quality. Unfortunately this resulted in the exclusion of several piglets due to not

meeting the inclusion criteria. This may have resulted in a bias towards piglets with lower grade lameness to be included in the study. The difficulty of getting enough valid measurements from very lame pigs may be a disadvantage for the practical use of the pressure mat as a method to identify lame pigs. However, pigs with severe lameness will easily be identified visually, without the need of additional pressure mat analysis.

Asymmetry indices

Symmetry is often considered a characteristic of normal gait. Although some asymmetry is considered normal in sound animals [25] , high asymmetry indices might indicate disturbances in the gait pattern. It has not yet been determined how much asymmetry can be considered normal and which variables best express gait symmetry and thus provide the best discriminators between sound and lame pigs. Therefore, in this study the differences in mean asymmetry indices were studied in both sound and lame piglets.

Since data from only 9 lame piglets were usable for analysis, the left/right distinction of the ASIswas left out of consideration and further analysis was performed on the absolute ASI values. Therefore, in the current study no discrimination was made between left and right sided lameness. This choice was supported by the findings of Lequang et al. (Lequang et al., 2010), who did not find any significant differences in left-right ASIsof stance time, relative stance time (stance percentage), peak vertical pressure and amount of activated sensors. In accordance Light et al reported a perfect symmetry in dogs between left and right limbs of healthy dogs (Light et al., 2010).

Asymmetry indices are considered to be least influenced by variables such as velocity, gender and weight because of the intra-subject calculation. However, the degree to which pressure mat temporospatial ASIsof pigs might be sensitive to these factors has never been studied. In this study we checked for influences of gender and velocity on the ASIsof the four pressure mat parameters. In accordance with a study on the influence of gender on gait characteristics in cats (Verdugo et al., 2013), in our study also no relationship was detected between the different ASIsand gender.

We did however find an effect of velocity on the ASI for step duration in control as well as in lame piglets. To the authors' knowledge, the relation between velocity and the ASI for step duration has not been described before. This result contrasts with previous findings by Oosterlinck et al. (Oosterlinck et al., 2010a) and Meijer et al. (Meijer et al., 2014a) who did not find a significant influence of velocity on ASIsof kinetic parameters. The influence of velocity on ASI for step duration may be of concern for the use of this parameter in pigs, since it is difficult to maintain a fixed speed over several trials without disturbing natural gait and weight distribution in this species.

Until now, limited data were available for the four spatiotemporal ASIsobtained from pressure mat analysis in pigs. ASI means of these measures were higher in lame piglets than in controls.

Mean stance duration asymmetry was increased in lame pigs at both the affected and unaffected side. This was expected from the results of the study by Mohling et al (Mohling et al., 2014). They found that lame sows had significantly longer stance durations in the limb contralateral to the lame limb, and Karriker et al (Karriker et al., 2013) who found significantly shorter stance durations in lame limbs of sows. This could have contributed to the larger stance duration asymmetry in lame piglets that were observed in our study.

The increased step duration asymmetry and stance duration asymmetry in lame animals' affected side corroborates with results from the kinematic study in lame sows by Stavrakakis et al (S. Stavrakakis et al., 2015). In contrast to the findings their study, we also found increased asymmetry in the lame animal's unaffected sides.

Stance percentage asymmetry was also increased in the affected and unaffected side. Not much is known about this particular variable. However, in trotting lame horses, accelerometer measurements showed that stance duration increased and swing duration decreased in both the affected and the contralateral limb (Buchner et al., 1995). This would imply that if an ASI was calculated for these horses, it would not be larger than that of sound animals. In our pigs we did see an increased asymmetry in the lame animals. This difference may be due to the fact that the horses had an artificially induced support phase lameness, whereas the pigs in our study often had disorders that affected both the support and the swing phase of the gait cycle. This may result in more pronounced differences between the lame and the sound limb.

ICC's

The intra class correlations between the piglets were poor. This indicates a large variation within individuals compared to the variance between individuals. In this study two mean asymmetry indices were calculated from a total of 8 valid contact points. Considering this finding, it might be of interest to use multiple asymmetry indices, and asymmetry indices of several parameters, obtained within the same run. By using an average, it remains inconclusive whether the variation of ASIsonly exists between runs or within runs as well. In this study absolute ASIswere used. Alternatively, when using original ASIs (either negative or positive numbers) variability within runs may be an indicator for lameness.

Conclusions

The aim of this study was to determine pressure mat analysis of temporospatial gait symmetry to be used as a practical diagnostic tool for lameness. Currently, the majority of veterinarians diagnose lameness by visual observation. Although observations might be very accurate in trained observers, studies show a low replicability between different observers (Waxman et al., 2008), even with standardized protocols (Main et al., 2000).

This highlights the need for a new, practical, objective and fast diagnostic tool. In several species, among which pigs, the use of force parameters obtained by pressure mat analysis shows promising results in detecting lameness. The variables considered here (stance duration, step duration, step length and stance percentage) are able to detect differences in mean ASI between lame and sound piglets. However, because of the use of absolute values of the average asymmetry indices this study remains inconclusive about the method's ability to discriminate between left and right sided lameness. Additionally, at least two proper contacts per limb, put down on the pressure mat in the right order are necessary to obtain an ASI. This excludes piglets with irregular gaits or extreme lameness in which the affected limb is not or hardly used. Currently, data-analysis is a time-consuming process because of the lack of software to automatically assign footprints to the correct limb for quadrupeds. Before the pressure mat is suited to be used in clinical situations or as a routine screening method for lameness in industrial pig farming, automatic selection of valid measurements, claw contact assignation and asymmetry indices calculations should be available.

Methods

The study was reviewed and approved by the local ethical committee of Utrecht University (DEC no 2012.III.05.04), The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

Animals

A total of 46 *Topigs 20* pigs was included in this study. The control group consisted of 24 healthy pigs (12 ♂, 12 ♀) ranging in age from 6 to 7 weeks supplied by a commercial breeding farm. The group of clinically lame pigs consisted of 22 three- to ten-week-old animals (12 ♂, 10 ♀). The pigs were transported to the animal facility of the Department of Farm Animal Health, Veterinary Faculty, Utrecht University, The Netherlands. The control group was delivered in one batch and was allowed to acclimatise for 1 week. The group of lame animals consisted of several batches and enrolled the experiments after one day of acclimatisation to the new environment. At the end of this study, the same pigs

were used in another study (Meijer et al., 2015) to assess the effect of pain relief on activity and gait. In that study, other criteria for the number of footprints per run were set, resulting in a different number of animals being included.

Housing

At arrival, within both groups, the pigs were randomly divided over 3 pens in the research facility of Utrecht University. All pens had closed concrete floor on which sawdust was provided as bedding material. The pens were similar in surface, ranging from 3.68 to 3.96 m^2, and contained no more than 8 pigs, providing them enough space according to EU legislation (0.35m^2/10-20kg pig). The animals were fed *ad libitum* (Groeiporco, De Heus Animal Nutrition, Ede, The Netherlands) and had *ad libitum* access to water. The ambient temperature in the stalls was 24°C. Additional heat lamps were provided if needed. The pigs were exposed to both daylight and artificial lighting from 7 a.m. to 6 p.m. (11 hours a day). Toys such as metal chains and plastic balls were provided during the entire experiment.

Data recording

Gait parameters were collected using a pressure mat (Footscan® 3D Gait Scientific 2 m, supplier: RSscan International, Olen, Belgium). The active sensor surface of this mat measured 195 cm X 32 cm, containing 16384 sensors (2.6 sensors per cm^2), with a sensitivity of 0.27-127 N/cm^2 and a sampling frequency of 126 Hz. A few modifications, as previously described by Meijer et al. (Meijer et al., 2014a), were made to the test setting in order to ensure the pigs' comfort and to prevent them from leaning against the walls. The mat was connected to a laptop with dedicated software (Footscan® Scientific Gait 7 gait 2nd generation, RSscan International, Olen, Belgium). The mat was calibrated according the manufacturer's instructions.

Quantification of lameness

To establish the absence or degree of lameness, both visual scoring and pressure mat analysis were used. Visual scoring was performed by a trained veterinarian, according to a modified version of the system validated by Main et al.(Main et al., 2000).

Pressure mat analysis provided information on stance duration, step duration, step length and stance percentage. Stance duration is defined as the time the limb is in contact with the ground surface, step length is the travelled distance between the right and left limb with step duration being the time needed for this distance. Stance percentage is the ratio between the time the limb is in contact with the ground compared to the swing phase of that limb.

Procedure

To minimize handling-associated stress, the pigs were tested in the order they presented themselves in. After letting an individual out of its pen, it walked freely and by itself to the testing area and into the holding pen at the beginning of the pressure mat. Pigs were allowed to explore the test apparatus and received a treat when they crossed the runway. The pig remained in the apparatus until 2 correct runs were collected, which never took more than 15 minutes.

A run had to fulfil the following criteria to be considered correct and to be included in the study: the pig had to trot the entire length of the runway in a straight line without stopping, looking straight ahead. Because the lame pigs appeared to be unable to trot, criteria for this group were adjusted by replacing the criterion trot for walk. The other criteria could still be met. All of these criteria were judged by two observers. Velocity was determined from the pressure mat data.

After recording the data for this study, the piglets performed in an additional study which took one extra day. After that study had been completed, the pigs were euthanized by first sufficiently sedating them with 2 mg/kg Azaperone IM (Stresnil, Elanco Animal Health, Greenfield, USA) followed by 200 mg/kg Pentobarbital IC (Euthanimal, Alfasan, Woerden, The Netherlands). Hereafter, the pigs were necropsied at the Department of Pathobiology of the Faculty of Veterinary Medicine of Utrecht University. Gross pathology confirmed the pigs' general health at the time of death. Specific attention was paid to the limb joints. They were dissected free and inspected for any macroscopic signs of joint disease. The pigs in the control group showed no macroscopic changes in any of the joints, in all the lame pigs clinical diagnosis was confirmed by macroscopic changes such as arthritis in the affected joint.

Data analysis

To ensure the reliability of the data, additional demands were set for the collected runs. If these were met they were considered a "valid measurement". Pigs with less than 2 completed correct runs and pigs with less than 8 contacts with the pressure mat (each claw twice) per correct run were excluded from the analysis. The data of 22 sound control pigs and 9 lame pigs met the criteria for inclusion in the data analysis (see Figure 2).

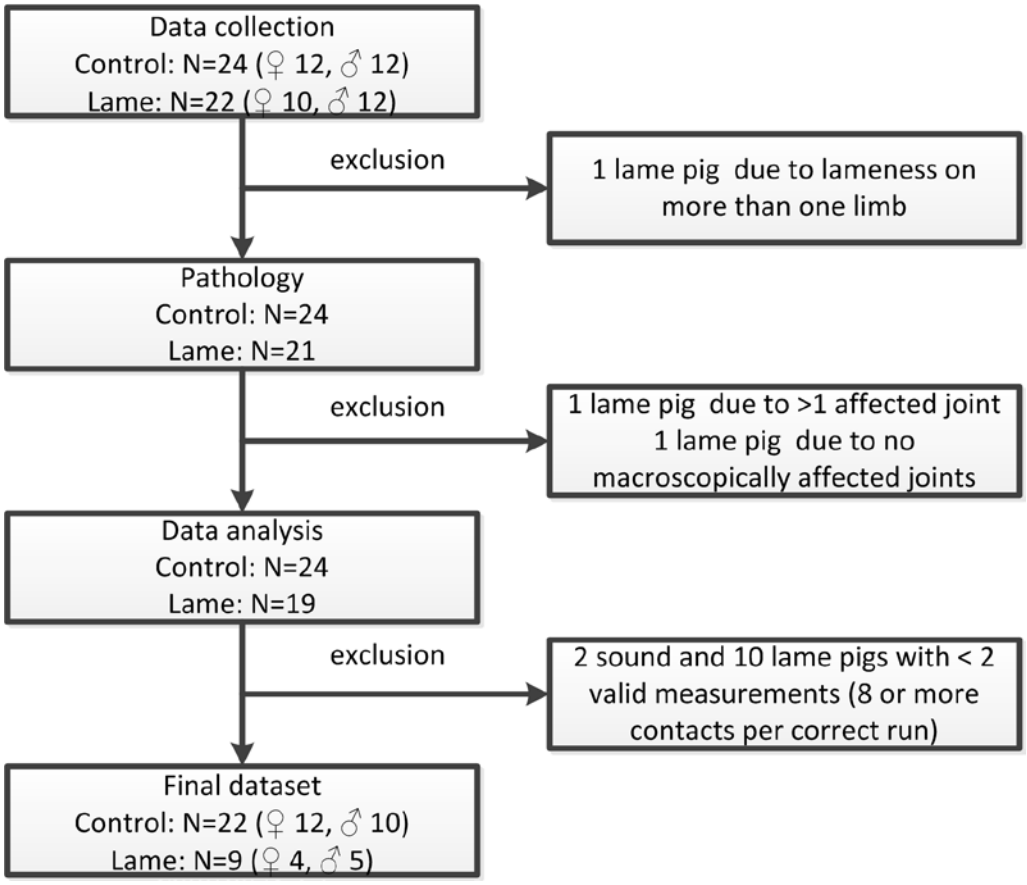

Figure 2. Animal selection process: display of inclusion and exclusion criteria of animals in this study.

Claw strikes from the 2 valid measurements per pig were automatically recognised by the purpose-built program Pawlabeling (Flipse, 2013) and manually assigned to the left fore (LF), right fore (RF), left hind (LH) and right hind (RH) limb. For every pig, means for step length, step duration, stance duration, and stance percentage were calculated for each claw in each of the 2 valid measurements. Fore and hind limb asymmetry indices (ASI) of all variables were calculated using the formula (Meijer et al., 2014a; Oomen et al., 2012)

$$ASI = \frac{L - R}{0.5(L + R)} * 100$$

where L= mean of left claw and R = mean of right claw.

Using this method, the ASI ranges between the values -200 and 200 with 0 indicating perfect symmetry. Positive or negative deviations indicate a higher loading of the left or

right limb respectively. For further statistical analysis, the absolute value of the ASIswas used, removing the distinction between right- or left-sided asymmetry because of the limited number of lame animals and the uneven deviation of left and right sided lameness among them. The final dataset contained 4 ASIsper variable per piglet, consisting of one front limb and one hind limb ASI per measurement for each of the two valid measurements. This results in 88 values for the sound controls and 36 values for lame piglets.

Statistics

All data had to be [$\log_{10}$ (y+1)]-transformed to meet normality assumptions and to avoid transformation problems as some piglets had asymmetry indices equal to 0. Consequently the estimates from the models are on the $\log_{10}$ scale and were back transformed by 10-power and should be interpreted as a ratio (multiplication factor) between the group (geometric) means. For further analysis, the data of lame piglets were subdivided into groups: ASIsoriginating from the affected and non-affected side (e.g. in case of forelimb lameness, the front side ASIswere considered as affected side data, hind side ASIsof that individual as non-affected side data). A linear mixed effects model was used to evaluate the effect of lameness, gender and velocity on the different dependent variables (ASIsof PVF, SD and VI)) with piglet set as random effect and group as fixed factor. The stance percentage could not be analysed with a mixed model and therefor a general linear model was used instead. To assess variability between the different measurements of front- or hind limbs of one piglet, intra-class correlations were calculated and interpreted according to Shrout and Fleiss (Shrout and Fleiss, 1979).

Data were analysed using SPSS statistics 21 (IBM) and SAS 9.4 (SAS institute Inc.), with statistical significance set at $p < 0.05$

Competing interests

The authors declare that they have no financial or non-financial competing interests.

Authors' contributions

MP transformed and analysed the data and drafted the manuscript. EM contributed to study design and data collection and critically revised the manuscript. IF contributed with the use of the purpose-built program 'Pawlabeling'. HV provided advice on the analysis, and critically revised the manuscript. FJS supervised the process, provided advice on data analysis and critically revised the manuscript. All authors read and approved the final manuscript.

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Appendix 2

Gait analysis of healthy dogs using a pressure-sensing walkway: influencing factors on asymmetry indices

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Under review

Abstract

Lameness is a common problem in veterinary practice. In order to evaluate treatment effects, objective methods to quantify gait are needed. Pressure-sensing walkways (PSW) are able to provide both kinetic and temporospatial information about gait, and offer some distinct practical advantages for use in clinical practice, compared to other methods such as force plates and kinematics.

Asymmetry indices (ASI's) comparing left- to right limbs have been used in several studies. Although they are very useful to quantify lameness in one limb, they may be less suitable when two limbs are affected, which may be the case in several conditions that affect both hind limbs. Ratio's comparing hind-to-front symmetry (pelvic-thoracic ASI's) may provide more information about bilateral hind limb dysfunction.

Regardless of the asymmetry index used, several factors are known to influence kinetic and temporospatial gait parameters. Limited information of the effect of these factors on left-right ASI is available, and virtually no data exist on their influence on pelvic-thoracic ASI's.

In this study we used 33 healthy, client-owned dogs to analyse the effect of side of guidance, handler (owner or unfamiliar handler), pace (walking or trotting), weight category and gender. We studied both left-right and pelvic-thoracic ASIs of three PSW-generated parameters: Peak Vertical Force, Vertical Impulse and Stance Duration.

Left- right ASI of Peak Vertical force was only influenced by pace, and no influences on left- right ASI of Stance Duration were found. Gender influenced left-right ASI of Vertical Impulse. Pace and weight category influenced pelvic-thoracic ASIs of all variables. Pelvic-thoracic ASIs of Stance Duration and Vertical Impulse were influenced by side of guidance, and pelvic-thoracic ASIs of Peak Vertical Force and Vertical Impulse by both handler and gender.

Both left-right and pelvic-thoracic ASIs may be affected by several variables. The magnitude of the effects is small from a clinical viewpoint, however they may become larger in lame animals. It is therefore necessary to account for the mentioned influencing factors when designing clinical studies or when using PSW's to assess treatment effect in practice .

Keywords: Gait analysis, symmetry, handler, weight, gender, dog, kinetic, temporospatial

Background

Hind limb lameness in dogs is a problem often encountered in clinical practice. The causes of hind limb lameness are diverse, but include hip dysplasia, rupture of the cranial cruciate ligament, patellar luxation and degenerative lumbosacral stenosis. In order to evaluate treatment and prevention strategies for these diseases, objective methods to quantify lameness are needed.

Several techniques to quantify lameness have been used, of which visual scoring, kinetic and kinematic gait analysis are used most often (Gillette and Angle, 2008). Visual scoring is fast and cost-effective, but it may suffer from inherent subjectivity, is affected by observer bias and has limited intra- and inter-rater agreement, especially in untrained observers and in rating patients with mild lameness (Keegan, 2007; Quinn et al., 2007; Waxman et al., 2008).

Kinetic analysis using force plates (FP) has extensively been used in veterinary medicine and is widely accepted as an objective tool to study normal gait and lameness (Gillette and Angle, 2008). It does, however, have some drawbacks. Because the FP cannot distinguish between consecutive footfalls, many runs are often needed to collect the required number of valid observations. Also, many kinetic parameters are influenced by factors such as subject velocity (Evans et al., 2003; Riggs et al., 1993; Voss et al., 2010)

Temporospatial parameters acquired by kinematics have been used in dogs as well (de Medeiros et al., 2011; Foss et al., 2013; Hogy et al., 2013), but their collection and analysis are time-consuming and expensive.

Pressure-sensitive walkways (PSW) are increasing in popularity as a tool for assessing locomotion, using both kinematic and temporospatial variables. They have been used to quantify normal locomotion, to detect lameness and to evaluate the effect of treatments in horses, pigs, cattle, dogs and cats (Kim and Breur, 2008; Lascelles et al., 2007, 2006; Meijer et al., 2014a; Oosterlinck et al., 2011; Oosterlinck et al., 2010a; Romans et al., 2004; Van Der Tol et al., 2003; Verdugo et al., 2013).

PSW's offer a number of advantages over more traditionally used FP. They allow recording of simultaneous, consecutive, and collateral foot strikes in a single passage over a walkway, requiring fewer trials and compensating for inter-trial variability compared with traditional single-stride force plate analysis. Because several footfalls may be recorded on the plate, they allow measurements of more diverse animal populations regarding size and breed (Lascelles et al., 2006). Another advantage is that they provide information about the distribution of forces within a pawprint. Furthermore, they are able to record a number of spatiotemporal gait variables, extending the possibilities to

evaluate dogs with neurologic diseases (Gordon-Evans et al., 2009). They are especially suited for use in clinical practice since they are portable, they do not need elaborate setups like kinematic analysis does and they are relatively cheap compared to other options.

Although PSW's offer some distinct advantages over traditional FP's, they share some of the drawbacks of FP's. Kinetic parameters of both PSW's and FP's are known to be affected by a number of factors, including velocity, and are therefore not always replicable (Evans et al., 2003; Meijer et al., 2014a; Oosterlinck et al., 2011; Riggs et al., 1993). In FP analysis researchers often try to minimise sources of variation by setting, for example, velocity within strict limits for runs to be valid. This may pose a problem when the research population is heterogeneous, or when lame animals are used that may not be able to walk comfortably at the required speed. Pressure mats offer an advantage because several footfalls may be recorded in one run, enabling the use of asymmetry indices (ASI's) to compare individual limbs within a run to each other. The recording of several footfalls in one run also means that less runs need to be done. This is especially important in lame animals that are unwilling or unable to walk for prolonged periods.

Previous studies on left- to right asymmetry indices (L/R ASI's) have found high diagnostic accuracy for detecting lameness in dogs (Fanchon and Grandjean, 2007; Oosterlinck et al., 2011). Recently, Vassalo et al (Vassalo et al., 2015) used L/R ASIs to evaluate locomotion in dogs with pelvic fractures. Considerably less attention has been given to pelvic to thoracic limb asymmetry (T/P ASI's). Previous results of FP analysis in dogs with degenerative lumbosacral stenosis (Suwankong et al., 2007) found an increase in the P/T ASIs of the propulsive forces after treatment, indicating that T/P ASI may be a useful parameter to study conditions that affect both hind limbs.

In order to use PSW as an objective tool to evaluate hind limb lameness in dogs, more information is needed. In this paper we evaluated the main influencing factors on asymmetry indices of three PSW variables: Peak Vertical Force (PVF), Stance Duration (SD) and Vertical Impulse (VI).

Materials and Methods

Animals

Thirty three healthy private owned dogs were included for measurement on the pressure mat (Table 1).

Table 1. Breed, Age (months), Gender and Weight (kg) of the healthy, client-owned dogs used in this study. F= Female, FN = Female neutered, M = Male, MN = Male neutered.

Breed	Age (Months)	Gender	Weight (kg)
Toy Poodle	7	F	2
Chihuahua	12	F	2
Miniature Poodle	8	F	4
Miniature Poodle	43	FN	4
Dachshund	28	MN	5
Dachshund	39	F	5
Miniature Poodle	37	F	6
Jack Russell Terrier	34	MN	7
Yorkshire Terrier	57	M	7
Jack Russell Terrier	94	FN	9
Cairn Terrier	144	F	9
Cavalier King Charles Spaniel	50	M	12
Spanish Waterdog	23	FN	16
Galgo Espagnol	60	FN	17
Standard Schnauzer	87	F	21
Galgo Espagnol	84	FN	22
Belgian Malinois	48	F	25
Belgian Malinois	132	FN	25
Crossbred	77	FN	26
Labrador Retriever	68	M	30
Weimaraner	23	MN	31
Labrador Retriever	84	FN	34
Doberman Pincher	82	MN	37
Golden Retriever	76	M	41
Bernese Mountain Dog	48	F	43
Bernese Mountain Dog	69	FN	50
Greater Swiss Mountain Dog	71	M	51
Portuguese Mountain Dog	37	FN	54
Portuguese Mountain Dog	83	FN	57
Newfoundland	19	F	59
Great Dane	35	M	62
Great Dane	33	FN	64
Newfoundland	86	MN	69

Health status was confirmed by a veterinarian (DA) by examination of possible orthopedic and neurologic symptoms involved in locomotion. Weight of the animals varied from 2 to 69 kg. Weight categories were defined < 5, 5 - <15, 15 - <30, 30 - <50 and ≥ 50 kg and each weight category consisted 6 to 7 dogs. Eleven males (5 neutered) and 22 females (12 neutered) were included. Dogs were from 24 (cross) breeds and varied in age between 4 and 144 months (median 48). Owners of the dogs were informed about the purpose of the measurements and owners gave orally permission to use the data for research.

Data collection

The gait analysis was performed using a footscan® 3D Gait Scientific 2 m system (RSscan International, Olen, Belgium) with an active sensor surface of 1.95 m × 0.32 m containing 16384 sensors with a sensitivity of 0.27-127 N/cm^2 and a measuring frequency of 126 Hz, connected to a laptop with dedicated software (footscan Scientific Gait 7 gait 2nd generation, RSscan International, Olen, Belgium). Measurements lasted up to a maximum of 2 seconds, after which the PSW stopped recording automatically. The PSW was calibrated according to manufacturer instructions before the start of the measurements and was positioned in between two rubber mats (1 m × 0.47 m) with sufficient space before and after the setup to allow for acceleration and deceleration.

The dogs were guided in a straight line over the pressure mat by either the owner or the veterinarian, guiding the dog on either the left or the right side. All dogs were measured both walking and trotting. The dogs were walked at their own preferred velocity. Velocity of each complete stride was calculated by dividing the stride length through the respective gait cycle duration. Only complete and separable paw measures were used for analyses. Three valid runs were measured per dog under 8 different conditions: pace (trotting/walking) at a convenient speed for the dog, with owner or veterinary technician and guidance at the left or at the right side. A run was considered valid when the run was in a straight line, with constant speed and correct pace.

Data processing

After data collection, it was determined which runs were included in the subsequent analyses, using a set of predetermined inclusion and exclusion criteria. Runs that deviated from a straight line, that had an obvious deviation from the expected gait cycle, or in which the registration by the PSW stopped before the dog reached the end of the plate were excluded. The remaining valid trials were processed using Pawlabeling (Flipse, 2013).Paw strikes were manually assigned their corresponding labels: left fore (LF), right fore (RF), left hind (LH) and right hind (RH). For each paw contact within a

run, validity was checked. Contacts were considered valid when they were fully on the plate and were completely within the 2-second-measurement timeframe.

Statistical analysis

For each limb, the kinetic variables PVF (N) and VI (Ns) were calculated. Furthermore, velocity (m/s), stance duration (SD) (ms), and duty factor (% of gait cycle) were determined.

For PVF, SD and VI Left/Right and Thorax/Pelvic ASIs were calculated:

$$\text{L/R ASI} = (\text{front left} + \text{hind left})/(\text{front right} + \text{hind right})$$

$$\text{T/P ASI} = (\text{front left} + \text{front right})/(\text{hind left} + \text{hind right})$$

The first 4 calculable L/R and T/P-ratios respectively are analysed because we collected at least 4 values per condition within each dog.

Statistical software R version 3.1.2 (R Development Core Team, 2008) was used for all analyses. A linear mixed effects model (Pinheiro et al., 2015) was used to analyze the outcome variable (L/R ASI and T/P ASI for PVF, SD and VI respectively) with explanatory variables side of guidance, person of guidance, pace, session, weight category and gender. The L/R ASI of the 3 outcome measures was log-transformed for the analysis. Dog was considered as random effect to take the correlated observations within dog into account. As the variability was different in the 5 weight categories, a variance function was added to the model to allow a different variance in each weight category. An autocorrelation parameter was added to the model for session within dog. Function dredge of library MuMIn (Bartoń, 2015) was used to calculate all competing models. The Akaike's information Criterion for small sample sizes (AICc) was used to select the best models. The parameter estimates with 95% confidence intervals of the final models are presented. The appropriateness of the models was evaluated by a visual inspection of graphs for normality and homoscedasticity.

Results

Based on AICc's the variables side of guidance, guiding person and weight category were removed from the final model for the L/R ASI (table 2). In PVF at walking pace the mean level of L/R ASI was 3 percent lower compared to the mean level at trotting pace. None of the variables affected the mean level of the L/R ASI of SD. VI was affected by gender with neutered animals having the largest effect (5% and 4% for neutered female and neutered male respectively).

Table 2. Estimates and 95% confidence intervals for intercept and rates with reference category in Left/Right asymmetry index for each of the explanatory variables in the final model for outcome variables Peak Vertical Force, Stance Duration and Vertical impulse. Variables side of guidance, guiding person and weight category were absent in all models.

	Peak Vertical Force			Stance Duration			Vertical Impulse		
	Estim.	95% CI		Estim.	95% CI		Estim.	95% CI	
Intercept*	0.96			1.00			0.99	0.95	1.03
Trotting***	1			-	-	-	-	-	-
Walking	0.97	0.95	0.99	-	-	-	-	-	-
Female***	-**	-	-	-	-	-	1		
Female N	-	-	-	-	-	-	0.95	1.90	1.00
Male	-	-	-	-	-	-	0.99	0.85	0.97
Male N	-	-	-	-	-	-	0.96	0.90	1.03

* Mean Left-Right ASI for reference category (left side, owner, 0-5 kg, female)
** Variable/category is not in the final model
*** Reference category

The mean T/P ASI was affected by most of the tested conditions for all 3 outcome measures (table 3). PVF walking pace (-0.13) and weight category (-0.34 and -0.22 for 30-50 kg and 50-100 kg respectively) had the highest negative effects on the mean T/P ASI but gender (+0.21 and +0.25 for Males and Neutered males respectively) caused the highest increase to the mean. Similar strength of effects could be observed for VI except that the effect of the weight category was stronger (-0.20, -0.64 and -0.53 for category 15-30, 20-50 and 50-100kg respectively). For SD the strongest effect was estimated by weight category (-0.08, -0.15, -0.21 and -0.21 for increasing weight categories). Side and person of guidance had a smaller effect on the mean T/P ASI compared to the effect of other conditions.

Table 3. Estimates and 95% confidence intervals (95% CI) for intercept and differences with reference category in Thoracic/Pelvic ASI for each of the explanatory variables in the final model for outcome variables Peak Vertical Force, Stance Duration and Vertical impulse.

	Peak Vertical Force			Stance Duration			Vertical Impulse		
	Estim.	95% CI		Estim.	95% CI		Estim.	95% CI	
Intercept*	1.68	1.51	1.84	1.26	1.21	1.30	2.05	1.86	2.23
Left side***	-**	-	-	0			0		
Right side	-	-	-	+0.01	-0.00	+0.03	+0.06	+0.02	+0.11
Owner***	0			-	-	-	0		
Technician	+0.05	+0.01	+0.09	-	-	-	+0.04	-0.01	+0.09
Trotting***	0			0			0		
Walking	-0.13	-0.17	-0.09	-0.03	-0.05	-0.01	-0.16	-0.21	-0.11
0-5 kg***	0			0			0		
5-15 kg	+0.11	-0.13	+0.35	-0.08	-0.13	-0.02	-0.03	-0.29	+0.23
15-30 kg	-0.00	-0.23	+0.22	-0.15	-0.20	-0.10	-0.20	-0.44	+0.04
30-50 kg	-0.34	-0.58	-0.11	-0.21	-0.26	-0.15	-0.64	-0.89	-0.39
50-100 kg	-0.22	-0.45	+0.01	-0.21	-0.27	-0.16	-0.53	-0.77	-0.28
Female***	0			-	-	-	0		
Female N	+0.03	-0.14	+0.21	-	-	-	+0.02	-0.16	+0.20
Male	+0.21	+0.00	+0.42	-	-	-	+0.17	-0.04	+0.39
Male N	+0.25	+0.04	+0.48	-	-	-	+0.28	+0.05	+0.50

* Mean T/P ASI for reference category (left side, owner, 0-5 kg, female)
** Variable/category is not in the final model
*** Reference category within condition

Variability of T/P ASI was large in light dogs (Figure 1) and decreased with increasing weight category. The mean T/P ASI was affected by more conditions than the mean level of L/R ASI for all outcome parameters.

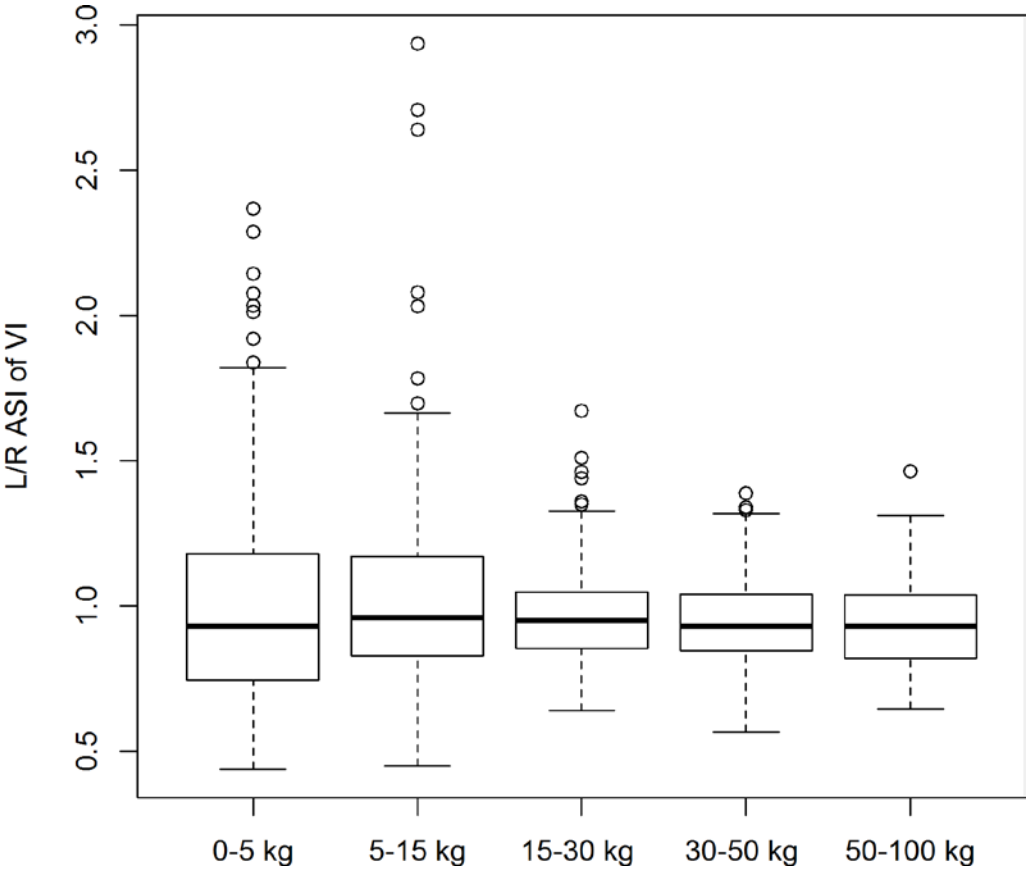

Figure 1. Example: Boxplots of left-right asymmetry indices of Vertical Impulse in weight categories (0-5 kg N=6, 5-15 kg N=6, 15-30 kg N=8, 30-50 kg N=6, 50-100 kg N=7). Each dot represents a measurement. Data are more variable in light dogs compared to heavy dogs.

Discussion

We investigated the effect of several conditions on L/R and T/P ASIs of PVF, SD and VI.

L/R ASI of PVF was higher at the walk than at the trot. No effect of pace on L/R ASI of SD and VI was observed. In ponies walking and trotting over a pressure plate, no influence of pace on front limb PVF and VI symmetry or hind limb PVF, SD and PVF symmetry was seen (M. Oosterlinck et al., 2011; Oosterlinck et al., 2010a). In a force plate study on experimentally induced lameness in Beagles, PVF forelimb asymmetry appeared higher at the walk compared to the trot, This impression was not confirmed statistically, because no statistical analysis on this difference was performed (Abdelhadi et al., 2012).

Although pace was visually confirmed as "walk" or "trot", duty factor was not available during data collection. Duty factor had to be calculated manually, which meant that it was not possible to check visual pace classification immediately. During analysis, some of the dogs that were classified as "trotting" were in fact, based on duty factor, walking and vice versa. However, since the results of this study are aimed towards clinical practice, we decided to use visual classification of pace instead of duty factor.

Force curves of walking dogs usually show a characteristic, double-peaked shape, whereas force curves at the trot only show one peak. In horses, this double-peaked shape was shown to be absent at lower velocities. The appearance of the double-peaked force curve was asymmetrical between contralateral limbs (Weishaupt et al., 2010). This asymmetry in force curve shape at certain velocities may also be present in dogs and account for the higher L/R ASI at the walk compared to the trot.

T/P ASI was lower in walking dogs compared to trotting dogs. In horses, the same asymmetrical development of a double-peaked force curve at higher walking velocities that is present between contralateral limbs, is also present between front- and hind limbs. Some animals first develop the double-peaked shape in the front limbs, others in the hind limbs. It is conceivable that this asymmetry is also resulting in different T/P ASI at the walk compared to the trot.

Leash side did not influence L/R ASI of any of the variables. Previous studies (Keebaugh et al., 2015) did find an effect of leash side on front limb symmetry. The lack of this effect in our experiment may be due to the way we calculated symmetry. Combining both front- and hind limb variables into one L/R ASI for all four legs may have obscured the effect of leash side on front limb symmetry, if this effect was not present in hind limbs. In the study of Keebaugh et al. (Keebaugh et al., 2015) only small dogs were included. In our experiment, dogs of different sizes were used. It is possible that smaller dogs experience more effect of leash side compared to larger dogs.

The absence of an effect of handler on L/R ASI of SD was in concordance with the findings of Keebaugh et al. (Keebaugh et al., 2015). We anticipated that dogs would look more often to their owner, resulting in an asymmetrical loading of the front limbs. This effect was not found in our study, indicating that for studies using L/R ASI several handlers can be used.

For T/P ASI, however, an effect of handler was found. T/P ASI of PVF and VI were higher when an unfamiliar person (the veterinary technician) was guiding the dog, indicating a relatively higher loading of the front limbs. It may be that the dog was looking up towards the owner more, and although this was not asymmetric towards the left or the right, it did cause some unloading of the front limbs, resulting in a change in front-hind symmetry.

Weight category did not influence L/R ASI of any of the variables. This is in agreement with previous studies comparing symmetry of pressure mat parameters in large and small dogs where no differences between groups were found (Kim et al., 2011; LeQuang et al., 2010a).

T/P ASI of all variables was influenced by weight category, with larger dogs generally displaying smaller T/P ASI's. Pressure mat analysis of small (mean 10.8 kg) and large dogs (mean 29.7 kg) showed that small dogs had a longer Stance Duration of the front limbs during the walk, this would translate as a larger T/P ASI. In a force plate study by Voss et al. (Voss et al., 2007) however, dogs with a lower body weight (mean 24.6 kg) had a higher PVF of the hind limbs compared to dogs with higher bodyweight (mean 35.5 kg). This would mean that the heavier dogs in that study would have relatively larger T/P ASI's, the opposite of what was found in the present study. In both the study by Voss and in the present study, several breeds with varying body conformation were used, which may have influenced front/hind distribution. It is possible that breeds with relatively broad chests and large heads have a relatively higher loading of the front limbs. Our study included for example a Cavalier King Charles Spaniel, a Chihuahua and a Dachshund, all of which have conformations that deviate from "general" dog conformation.

The larger variability of T/P ASI in lighter dogs may have been due to the width of the pressure mat. The active sensor surface was 0.32 m wide, which meant that very large dogs had to walk in a meticulously straight line in order for the run to be considered correct. In small dogs, although the runs were visually checked, there may have been some more room for deviations from a straight line.

Although the effect of gender on several human gait kinematics has been shown (Cho et al., 2004; Ferber et al., 2003), this effect was not present in pressure mat measurements of cats (Verdugo et al., 2013) or kinematic analysis of foals (Denham et al., 2012).

In the present study, a group of dogs with a very wide range of body weights was examined. Although breed was recorded, no morphometric measures were taken. This meant that even within the weight categories, body conformation could vary widely and we did not correct for these differences. Breed and body conformation have been shown to influence kinetic parameters in both dogs and horses [1–4], and they may influence T/P ASI as well as variability in kinetic and temporospatial parameters. Dynamic scaling (Hof, 1996) has been used to normalize gait data of dogs of various sizes (Voss et al., 2010) and may provide a method to reduce conformation-related variability.

Although significant influences of several factors were found, the question remains whether the magnitude of these influences is clinically relevant. In a study using force plate analysis to assess asymmetry in clinically lame dogs a cutoff left-right asymmetry of PVF of > 9% at walk and >6% at trot was able to distinguish lame dogs from sound dogs with a sensitivity of 0.63 and 0.90 respectively (Voss et al., 2007). This means that even in clinically healthy dogs some asymmetry is present. Since the magnitude of the influences of the factors we analysed is small, the relevance of these influences in healthy dogs is unclear. However, it is possible that in lame dogs some of the influences of these factors are accentuated.

Conclusions

When designing clinical studies using pressure mat analysis of dogs, several factors should be taken into account, depending on the outcome parameters used. Pace, leash side, handler, weight category and gender may all influence outcome parameters and should be corrected for.

List of abbreviations used

FP	Force plate
PSW	Pressure-sensitive walkway
ASI	Asymmetry Index
L/R ASI	Asymmetry index comparing both left limbs to both right limbs
T/P ASI	Asymmetry index comparing both front limbs to both hind limbs
PVF	Peak Vertical Force
SD	Stance Duration
VI	Vertical Impulse
LF	Left front limb
RF	Right front limb
LH	Left hind limb
RH	Right hind limb

Competing interests

DA is the owner of Aharon Orthomanuele Diergeneeskunde, a veterinary practice that specialises in orthomanual therapy for dogs and that uses a PSW to evaluate treatment success.

Author's contributions

EM drafted the manuscript. JV performed the statistical analysis. DA recruited the patients and performed the clinical examinations. IF built the program Pawlabeling to manage the raw data and perform the preliminary analysis. FJS provided advice on the writing of the manuscript. All authors read and approved the final manuscript.

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Appendix 3

The use of accelerometers to detect lameness in weaned piglets

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Under review

Abstract

Lameness in pigs is a highly prevalent problem, impairing animal welfare and causing economic losses. Lameness is difficult to detect because behavioural observations for detecting lameness are time consuming and subjective. An additional problem is that pigs are usually housed in large groups, impeding observation of individuals. The development of an objective method to easily measure activity in pigs would improve detection of lameness and help to develop better evidence based treatment options.

The aim of this study was to develop the use of accelerometers as a non-invasive method for assessing activity and easily detecting lameness in group housed, weaned, Topigs 50 piglets. The first phase was to analyse the behaviour of the piglets with and without the accelerometers attached dorsally between the shoulders. In particular, we evaluated the practical utility of a dog harness to hold an accelerometer in group-housed animals and whether the attachment of accelerometers would impact the pig's behaviour. Piglets were videotaped before, immediately after attaching, and 24 hours after attaching the dog harnesses with accelerometer. The second phase was to compare the activity of lame and sound piglets. In the third phase, the effects the administering a Nonsteroidal Anti-Inflammatory Drug (NSAID) on the activity of lame piglets was assessed.

Video footage 24 hours after attachment of the accelerometers showed that behaviour of the piglets was not influenced by the attached accelerometer. Activity intensity was not significantly different between lame (Mean±SEM, 4.77±0.03) and non-lame (4.73±0.02; $t28=-0.278$, $P=0.688$) piglets and before and after treating lame piglets with an NSAID (untreated, 4.17±0.63; treated, 4.35±0.65; $t12=0.645$, $P=0.950$). During 7.2% of the observations piglets lost their accelerometer.

We conclude that activity measured with accelerometers according to the described methods cannot be used to detect lameness in group-housed, weaned piglets.

Highlights

- Accelerometers were used to determine activity intensity of weaned piglets.
- Activity intensity was not significantly different for lame and sound piglets.
- Meloxicam had no significant effect on activity intensity of lame piglets.

Keywords: lameness, activity intensity, pigs, accelerometers, group-housing, NSAID, Meloxicam

Background

Lameness is a serious problem on pig farms. According to a British study nearly 20 percent of the weaned piglets kept indoors are lame (KilBride et al., 2009). The percentages of lame pigs among maiden gilts, pregnant gilts and pregnant sows are 11.8%, 14.4% and 16.9% respectively. In Belgium 15% of the sows are culled because of feet and leg problems (Pluym et al., 2013).

The economic loss due to the high prevalence of lameness is high on pig farms. Infectious arthritis, trauma, or osteochondrosis causing lameness of finishing pigs contribute to profit loss due to early culling, costs of medical treatment, a higher feed conversion ratio and a lower end weight of the pigs (Jensen et al., 2012; Willgert, 2011). Lame sows are culled after on average 2.6 parities while most sows are culled after on average 4.0 parities. Because sows are the most productive between their third and sixth parity, lame sows, on average, are culled before they reach their top of productivity (Pluym et al., 2013).

Lameness is also an indicator for compromised animal welfare because one can assume that the majority of lame pigs experience pain. However, not all animals with a disturbed locomotion are in pain. For example, animals with osteoarthrosis can be lame because they have mechanical restrictions, without being in pain (Kidd et al., 2001). However, their welfare is still affected, because lame animals have more problems with walking to the feeding trough and are more easily run over by the other animals. They also have a bigger chance of developing skin lesions and urinary tract infections (Heinonen et al., 2013).

In summary, because of its high prevalence, the economic losses, and the impact on animal welfare, lameness is a major problem on pig farms. To address this problem, more information on lameness in pigs is needed. It is very difficult to examine a pig and diagnose lameness. Pigs will vocalize loudly when handled and have a muscular body, which makes it difficult to hear or feel crepitation. Also, they will not walk in a steady gait, complicating the examination of locomotion. This leads to underdiagnosing this condition: lameness is often not recognized as a serious problem. Early diagnosis is very important to start treatment and prevent more economic loss and suffering of the animals, and perhaps even to adjust breeding to minimize the incidence of OCD.

On dairy farms, weekly locomotion scoring of all cows by a veterinarian and immediate treatment of the lame animals decreased the prevalence of lameness compared to visual detection by the farmer and treatment only when the farmer thought it would be necessarily (Gundelach et al., 2013). However, the diagnosis of lameness in pigs under field conditions is challenging. Often lameness is not recognized by the farmer. Farmers

and veterinarians usually score locomotion visually to detect lameness, but this method is time consuming and requires training to score reliably. Diagnostic methods like radiology and ultrasound cannot be used routinely under farm conditions and are very time consuming and expensive. Measuring activity could be an interesting tool to diagnose lameness and compare intervention methods like medication or prevention measurement. An easy, objective method to measure lameness can help research on lameness in weaned piglets or pigs in general.

A new research tool might be to use accelerometers attached to the animal's body. Accelerometers register movement by measuring acceleration and gravity. Accelerometers contain silicon beams that deform during acceleration or under the influence of gravity. This deformation is translated into voltage output, proportional to the applied acceleration. For each axis a beam measures acceleration. Accelerometers have been used to detect motion in goats (Moreau et al., 2009), cats (Lascelles et al., 2008), dogs(Brown et al., 2010; Hansen et al., 2007; Michel and Brown, 2011; Ryan Morrison et al., 2014; Yam et al., 2011), cows (Nielsen, 2013)(Sepúlveda-Varas et al., 2014) and sows (Ramonet and Bertin, 2015). Because moving is painful or difficult for a lame animal, it may show less activity. Lame dairy cows show less activity measured in steps per hour than non-lame cows. (Reader et al., 2011) Accelerometers are validated as a tool to detect lameness by measuring the activity or time budgets of cows [18]. Lame animals are expected to spend more time lying down and thus to show less activity. To our knowledge, no scientific proof for this expectation has yet been published. For convenience, weaned piglets are used in this study as a model for group-housed pigs. It is important for the accelerometer data to be representative for the activity of the piglets and that the piglets are not disturbed in their normal behaviour by wearing an accelerometer. Also, if the piglets are disturbed in their normal behaviour by, for example, shaking or scratching the dog harness it seems unlikely that the accelerometer delivers reliable data that show differences in activity level between lame and sound piglets.

We investigated the practicability and usability of measuring activity in group house piglets using accelerometers in three successive phases of a study. In the first phase the behaviour of the piglets before and after attaching the accelerometers was evaluated to see whether the presence of the accelerometers attached to the dog harnesses influenced the piglets' behaviour. In the second phase we compared the activity of lame piglets with that of sound piglets. In the third phase, the effect of Nonsteroidal Anti-Inflammatory drugs (NSAID's) on the activity of lame pigs was assessed. Lameness in pigs is often treated with painkillers or antibiotics. If lame pigs are less active, then the treatment of lameness with pain medication would increase the activity of lame pigs. Nonsteroidal anti-inflammatory drugs (NSAID's) such as Ketoprofen or Meloxicam improve the gait of

lame pigs, but it has not been shown yet to improve the activity of the pigs (Mustonen et al., 2011). Using accelerometers, the activity of lame piglets was evaluated before and after treatment with Meloxicam in order to find an increase in activity after treatment.

The main focus of the present study was to develop the use of accelerometers as an animal-friendly, easy to use tool for detecting and diagnosing lameness in a research setting. We expected that lame pigs were less active than sound pigs and that treatment with an NSAID would reconstitute normal activity levels.

Results

With 52 piglets and a total of 69 observations of 6 hours plus 30-60 minutes habituation, four accelerometers came loose and one accelerometer was almost loose (7.2%). 59 observations had reliable data to use for analysis.

Phase 1: Utility of accelerometers

Because the number of observed subjects is small (n=4), only descriptive statistics were performed on these data. Lying on the belly and lying on the side are merged as lying down because the piglets spent little time lying down during the observation period. Other behaviours registered were locomotion, exploring, social interaction, shaking and aggression. Plots of the proportion of time the piglets performed certain behaviour showed that the piglets' behaviour altered after attaching the dog harnesses with accelerometers. 24 hours after attaching the harness the proportion of time the piglets spent showing these behaviours was similar to the proportion of time before the harnesses were attached (data not shown). On all video recordings a pen mate pulling on the harness was observed at some time point. This behaviour, however, did not affect the accelerometer data as this did not increase fluctuation in the data. When the images of a penmate pulling the dog harness were observed more carefully, we noticed that the accelerometers stayed on the right place without much movement when a penmate pulled at the dog harness.

Phase 2: Difference between lame and non-lame animals

Average Activity Intensity (AI)

Although this measure was close to the diagonal line in the normal Q-Q plot, according to the Shapiro-Wilk test average AI had no normal distribution (P=0.008). After Log_{10} transformation, the average AI were normally distributed (Shapiro-Wilk test: P=0.082, 95%) (fig. 1). The 95% confidence interval of average AI of lame piglets was 2.47-4.22

and of non-lame piglets 2.77-4.21. Lame and non-lame piglets did not differ for the log-transformed average AI (t_{28}=-0.278, P=0.688).

Maximum AI

This measure was close to the diagonal line in the Normal Q-Q plot, but according to the Shapiro-Wilk test maximum AI was not normally distributed (P=0.041)(fig. 1). The 95% confidence interval of maximum AI of lame piglets is 36.64-56.77 and of non-lame piglets 34.31-50.47. Log_{10} maximum AI had a normal distribution (P=0.865). The maximum AI was not different between the lame and non-lame piglets (t_{28}=0.726, P=0.455).

Part active

Normality of part active was confirmed by the Normal Q-Q plot and the Shapiro-Wilk test (P=0.066) (Fig. 1). The 95% confidence interval of part active of lame piglets is 0.60-0.74 and of non-lame piglets 0.67-0.77. Lame piglets and non-lame piglets did not differ for part active (t_{28}=-1.306, P=0.202).

Average intensity

Average activity was close to the diagonal line in the Normal Q-Q plot. Normality was confirmed by the Shapiro-Wilk test (P=0.107) (fig. 1). The 95% confidence interval of average intensity of lame piglets is 2.47-4.22 and of non-lame piglets 2.77-4.21. Lame piglets and non-lame piglets did not differ for average intensity (t_{28}=0.083, P=0.934).

Phase 3: Effect of Meloxicam on the activity of lame piglets

Average AI

The Log_{10}-transformed average AI was not different before and after administration of Meloxicam (t_{12}=0.645, P=0.950) (fig. 2). The 95% confidence interval of average AI before the administration of Meloxicam was 2.94-4.22 and after administration of Meloxicam 2.47-4.42.

Maximum AI

No significant difference were found in maximum AI before and after the administration of Meloxicam (t_{12}=0.260, P=0.203) (fig. 2). The 95% confidence interval of maximum AI before the administration of Meloxicam was 43.47-69.56 and after administration of Meloxicam 32.43-55.50.

Part active

Similarly, the part active was not influenced by the administration of Meloxicam (t_{12}=-0.837, P=0.419) (fig. 2). The 95% confidence interval of part active before the administration of Meloxicam was 0.62-0.78 and after administration of Meloxicam 0.60-0.76.

Average intensity

The same picture was found for average intensity. Administration of Meloxicam did not affect this measure (t_{12}=-0.349, P=0.733) (fig. 2). The 95% confidence interval of average intensity before the administration of Meloxicam was 4.60-7.44 and after administration of Meloxicam 3.82-5.96.

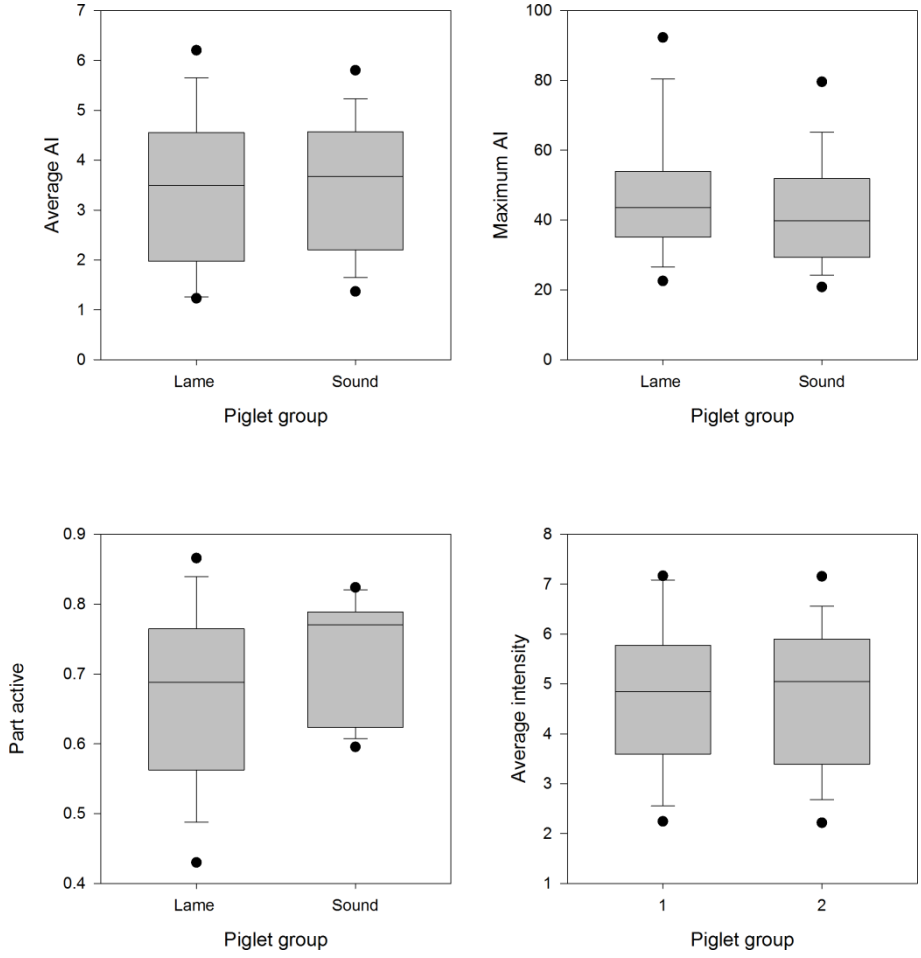

Figure 1. Boxplot of average, maximum AI, part active and average intensity of lame and sound piglets. Average AI and maximum AI were log10-transformed before analysis.

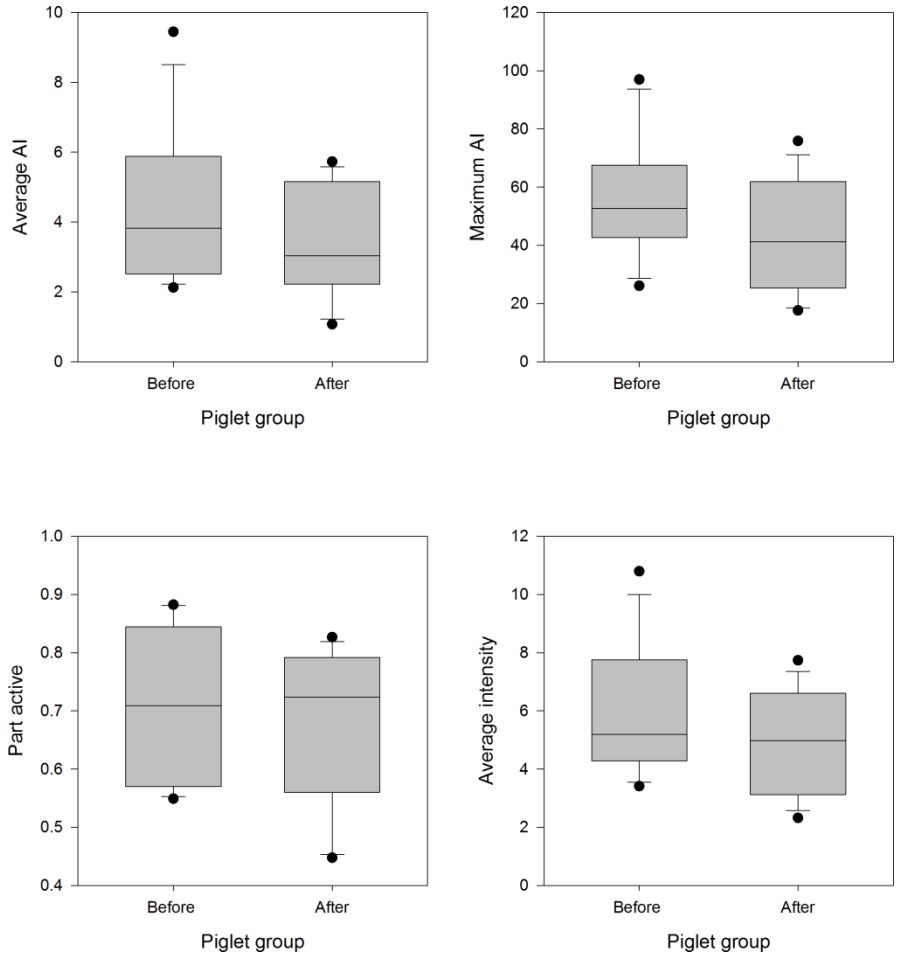

Figure 2. Boxplot of activity parameters before and after the administration of Meloxicam.

Boxplot of average AI, maximum AI, part active, and Average intensity before and after the administration of Meloxicam, a NSAID. Average and maximum AI were log10-transformed before analysis.

Discussion

The aim of this study was to evaluate accelerometers as a non-invasive diagnostic method for lameness in pigs. The harnesses with accelerometer themselves did not disturb the piglets after a short habituation period. The tested piglets only paid attention to the harnesses when pen mates pulled on the harnesses. The interest of the pen mates for the harnesses did not decrease over the 24 hours during which the piglets wore the harnesses. Presentation of distracting toys and Lucerne in the pens did not affect the interest of penmates to manipulate the harness. It is unknown if the piglets will have less interest in the accelerometer harnesses after 48 hours, like they do with a rope (Trickett et al., 2009a)). The pen mates pulled and bit the harnesses and because the test subjects tried to shake their pen mates off they were obviously disturbed by their pen mates. Results of a pilot study with cow pedometers attached to sows also showed that it is difficult to use equipment attached to the body of a group-housed pigs (own, unpublished observations). The pen mates bit the equipment and the securing material until it came loose. In the present study, most accelerometers stayed secured to the piglets but that was probably because the piglets are less skilled and have smaller teeth than the sows. This indicates that the behaviour of group housed pigs and piglets will always be influenced by the reaction of their pen mates on the attached equipment. Consequently, the data will be influenced by the other animals of the group, reducing the reliability of measuring the activity of the focal animal. In this experiment the influence of pen mates was reduced by providing extra distraction material but when longer recording periods are used the distraction material may become less interesting for the pen mates.

The accelerometers do not directly measure movement but acceleration. This means that piglets with less variation in their activity pattern will seem less active regardless of whether they are moving or standing still. The acceleration also cannot discriminate between moving fast and moving slowly. However, because the piglets are kept in small pens and the unsteady natural gait of the animals, the accelerometer data seem to be a good indicator of the activity distribution of the animals. This was also confirmed by the video observations.

A cut-off value for the standard deviation to determine whether the piglets are active or inactive, as described by Bai et al. (2014) has not been used in the present study. A clear drop in frequency of standard deviations, where the standard deviations can be divided in high “active” or low “rest” was not observed. The standard deviation of a 20- to 30-minute resting period was used instead to differentiate between "active” and “non-active”. This difference can be caused by the lower sampling frequency of the accelerometers. Bai et al. (2014) used a sampling frequency of 10 Hz. To collect data for 6 hours straight, we used the low sampling frequency of 1 Hz. Consequently, the resolution

of measurement during 6 hours was lower than that of Bai et al. (2014). Because of the short battery life measuring periods could not be extended. Other studies that compare activity levels in dogs used longer sampling times of seven hours (Hansen et al., 2007), 17 hours (Morrison et al., 2014), three consecutive days (Morrison et al., 2014) or seven days (Brown et al., 2010; Yam et al., 2011). Studies in cows used 40.9 hours (Cook et al., 2007) or 48 hours (Cook et al., 2008) and a study in sows used 24 hours (Grégoire et al., 2013). In the present study, data were collected when the piglets were most active. It remains to be studied whether there are more differences in activity levels of lame or sound piglets during the less active periods of the day.

As is common practice in industrial pig farming, the piglets had no place to hide from their pen mates. This may be a reason why the activity in the lame piglets did not decline. The pen mates stimulated the lame piglets to move despite the pain they might experience. Piglets in less crowed pens spend more time lying down than piglets in more crowded pens. The percentage lame animals was not different for more or less crowded pens.(Vermeer et al., 2014)

Lame dairy cattle in stables with more bedding on their matrasses show a stronger decline in activity and spend more time in the stalls compared with lame cows kept in stables with sand bedding(Cook et al., 2008). The piglets used in our study had partially slatted and partially concrete flooring. No bedding material was available for more comfort. This could have contributed to the unchanged activity of the piglets.

Since the piglets may be disturbed by the pen mates pulling the harnesses and some piglets were able to get rid of their accelerometer it was decided to use a short habituation period of between 30 and 60 minutes, before the accelerometers started collecting data. The behavioural analysis of the first group of piglets showed that after 24 hours their behaviour had returned to normal. It is uncertain whether the habituation period was sufficiently long to accustom the piglets to the harnesses.

Conte et al. (2014b) showed that a short stride length (<83 cm) or a low speed can indicate that a sow walked individually at a steady pace is lame but these parameters have a low sensitivity (Conte et al., 2014b). Measuring activity intensity of lame piglets may encounter a similar problem. A severely lame piglet will likely show less activity but not every lame piglet is less active. This could make measuring activity an unreliable diagnostic method to detect lameness.

Because the piglets received extra distraction material after attaching the harnesses, the observed behavioural changes can also be caused by the extra toys. When weaned piglets receive an extra rope as environmental enrichment the proportion of time the animals are in contact with the rope decreases already after two days (Trickett et al., 2009b).

Whether they already lose interest in the rope within 24 hours is unknown. Even if they stay interested in the extra rope it is uncertain if a rope is enough distraction to change the behaviour of the pen mates towards the accelerometer harnesses.

Conclusions

Activity intensity measured with accelerometers did not detect differences between lame and non-lame piglets. Also, treatment of lame piglets with Meloxicam did not affect activity intensity. In the field it is not practical to attach accelerometers to all animals, especially not when they are group housed. This study suggests measuring activity with accelerometers is not a reliable tool for detecting lameness in piglets. The activity of commercially kept, group-housed, weaned lame piglets shows no decrease according to accelerometer data. Other, sensitive methods to easily detect lameness in group-housed animals need to be found.

Methods

The study was reviewed and approved by the local ethical committee of Utrecht University (DEC no 2013.III.12.088), The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering. The study was internally funded by the Department of Farm Animal Health of Utrecht University.

A total of 69 3.5- to 8.5-week old, piglets (Topigs 50) were selected from the pigs of the farm of the Faculty of Veterinary Medicine of Utrecht University. The piglets were born on this farm and were all weaned at approximately 4 weeks of age. They were housed in pens with a partially slatted and partially concrete flooring, 3.80 meter deep and 1.20 or 2.20 meter wide, depending on whether 10 or 20 piglets were kept in the pen. The stable was illuminated by daylight and by artificial light (light on for 11 hours a day). All piglets had ad libitum access to water and food. Until 10 days after weaning, the piglets received weaning feed (speenkruimel 3691, De Heus Animal Nutrition, Ede, The Netherlands). Then, they were fed growing feed (big pr kruimel 3697, De Heus Animal Nutrition, Ede, The Netherlands). All piglets were vaccinated against circovirus and mycoplasma.

Distraction material

During preceding research with pedometers attached to sows, we observed that the sows removed the pedometers by chewing on them and on the securing material. Therefore, the piglets in the present study received extra ropes and Lucerne after securing the accelerometers and before the start of the measurements in their pens, in addition to the chains and chewing sticks that were already available.

Visual lameness scoring

Piglets were scored as lame or non-lame based on the lameness scorings system of Main et al. (2000c). This scoring system uses the gait, standing posture, behaviour of an individual within the group and initial response to human presence to identify lameness in group-housed pigs. The item “Pig's response after opening gate” from this original scorings system was not used in the selection protocol because it is time consuming to perform on each piglet. Piglets with a lameness score 0 were categorized as “non-lame” and piglets with lameness scores 2 or 3 were categorized as "lame”. Piglets with other lameness scores were not included in the study. When in doubt about its gait score, a piglet was not included in the study. Piglets with score 4 or 5 only stand up if they are strongly motivated or they are not able to stand up witch makes it self-evident that the accelerometers will show that the animals are less active. These lame piglets are also easier to detect by visual inspection of the pen. The difference between score 0 and 1 is also very small, which makes reliable scoring difficult. Moreover, piglets with score 1 may not experience enough pain to affect their activity. This scoring system has a 94% level of agreement when used by trained observers (Main et al., 2000). The gait was observed during several minutes. In the field, farmers don’t have enough time to observe the gait of every individual piglet. This makes this method unworkable for daily use by a farmer but it is possible to find lame piglets in a research setting.

Selection of subjects

During a period of 2 months the weaned piglets were visually examined twice a week to identify lame piglets, scored according to the method of Main et al. (2000). For the first phase of our study, 5 non-lame piglets were used. For phases 2 and 3, 16piglets with lameness score 2 or 3 were included in the study, If available, a non-lame pen mate of the chosen lame piglets was also equipped with an accelerometer, until also 16 non-lame subjects were tested. If a penmate was not available a piglet of a different pen but in the same stable and of approximately the same age was used. All piglets were moved to the weaned piglet pens at least 2 days before testing started to allow establishing a hierarchy between the pen mates and acclimatization to the new housing conditions. If the lameness was progressive and reached the score 4 or higher a piglet was excluded from this study and received treatment according to the treatment protocols of the farm. If a subject lost its accelerometer during the testing period the accelerometer data of that piglet during that period were not used for analysis. The selected piglets stayed in their home pens when the accelerometers were recording.

Accelerometers

The accelerometers used were the HOBO Pendant G Acceleration data loggers, from Onset Computer Corporation. They are tri-axial accelerometers, weighing 18 g and measuring 58 x 33 x 23 mm. With a logging interval of 1 hertz they can record data during a maximum of six successive hours. After six hours the data logger stopped recording, the dog harnesses were removed, and the logger were read out with an Optic USB Base Station. The accelerometers were tightly secured to an adjustable nylon dog harness with Vet Wrap and leucoplast on the back, between the shoulder blades (fig. 3) and the piglets stayed in their home pens with the penmates. The data loggers were recording from 10:00 a.m. until 4:00 p.m. This recording period was chosen because it has been shown that pigs in a "Comfort Class" housing system have activity peaks around 9:00 a.m. and 5:00 p.m. (Vermeer et al., 2014). They are also more active between these peaks than during the night so the piglets should be active during the recording time. The accelerometer data were plotted, using HOBOware Pro software.

Figure 3. The Hobo Pendant G data logger and the dog harnesses. The upper left panel shows one of the nylon, adjustable dog harnesses used. The upper right panel shows two piglets wearing a dog harness with an accelerometer attached, the blue arrows show where the accelerometers are. The lower panel is a photograph of the accelerometer used for this study.

Phase 1: Utility of accelerometers

For the first phase of the experiment 4 non-lame, 8-week-old, female piglets, were equipped with a dog harness with an accelerometer. The piglets were video recorded before, immediately after and 24 hours after receiving the dog harnesses for one hour. Their behaviour was scored using JWatcher (Blumstein and Daniel, 2007). The proportion of time in sight was calculated for frequently occurring behaviours to see if the behaviour of the piglets changed after attaching the harnesses.

Phase 2: Difference between lame and non-lame animals

Sixteen non lame piglets (9 female, 7 male, 4-8.5 week old) and 14 lame (6 female, 8 male, 3.5-6 week old) piglets were equipped with a dog harness with an accelerometer 30-60 minutes before recording. All piglets were recorded once.

Phase 3: Effect of Meloxicam on the activity of lame piglets

13 lame piglets (7 female, 6 male, 3.5-8.5 week old) were equipped with a dog harness with an accelerometer 30-60 minutes before recording. After recording, the dog harnesses were removed, the data loggers were read out and the piglets received 0.4 mg/kg Meloxicam (Novem 20 mg/ml, Boehringer Ingelheim) intramuscularly. The next morning the piglets were equipped with the same dog harnesses with the same accelerometers as the day before. The Meloxicam would then have its effect on the pain sensation of the piglets. When the accelerometers had collected data on the second day, the dog harnesses with accelerometers were removed.

Statistical analysis

Data were analysed using RStudio version 0.98.978 and IBM SPSS statistics version 22.

Activity Intensity

The data loggers collected data of the acceleration on three axes with a frequency of 1Hz, i.e. for every second three accelerations in g were logged. The raw accelerometer data were transformed to 'Activity Intensity' (AI) data according to a method described by Bai et al. (2014). For every 10 measurements the standard deviation was calculated. The activity intensity is a ratio of the resting standard deviation and the standard deviation of 10 successive measurements minus the resting standard deviation (the cutoff point). Activity intensity during a resting period was 0. During an active period the activity intensity exceeded 0. This method uses an estimated cut-off value for the standard deviation to decide whether the subject was active or in-active. A frequency peak in lower standard deviations can be found, when the frequencies of all the standard deviations are

plotted in a histogram. The standard deviation after this peak is the cutoff point for an active or in-active subject.

Because the frequency peak of lower standard deviations of the accelerometer data of the piglets was not very clear (see fig. 4) the mean standard deviation of 30 minutes of the longest successive resting period was used to calculate the cutoff point. The shortest resting period of a piglet was 20 minutes. For each 6-hour measuring period a resting period of 30 minutes was identified, inspecting the plots of the accelerometer data. In every graphic, periods where the three lines of the different axes showed the least variation were marked. The behaviour of one piglet was scored during the 6 hour video recording period. The video recording showed that, during periods with less variation of all three axes, the piglet was either standing still or lying down. The deviation in the accelerometer data during these periods were considered as representing noise. The average standard deviation (fig. 5) during a resting period was used as a cutoff point to determine AI. For each piglet this cutoff point was calculated. Residual movement and acceleration in the resting periods was caused by, for example, the breathing of the animal.

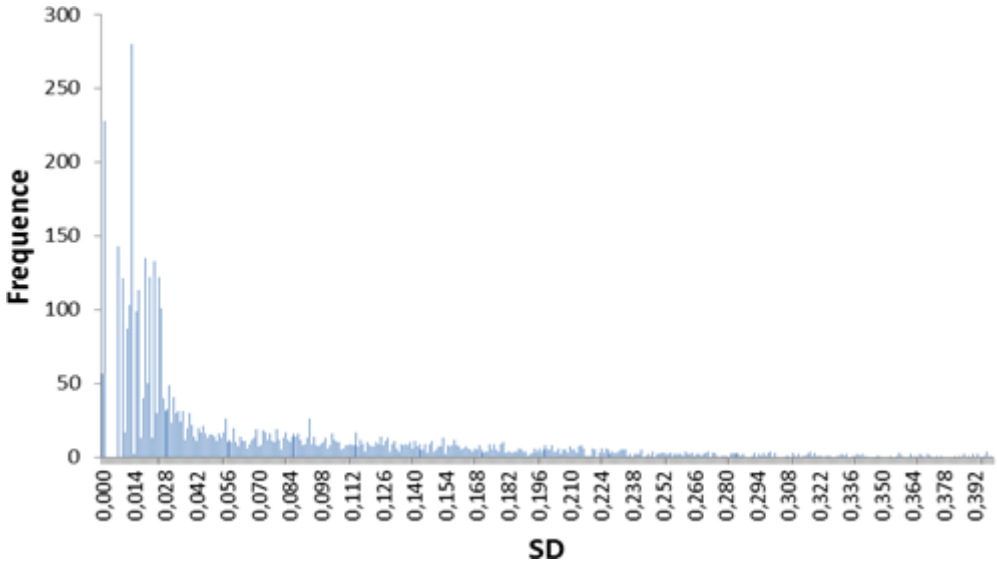

Figure 4. Histogram of the frequency of standard deviations of piglet 55.

No clear cutoff point to distinguish SD accelerometer data in rest and SD accelerometer data in action can be found.

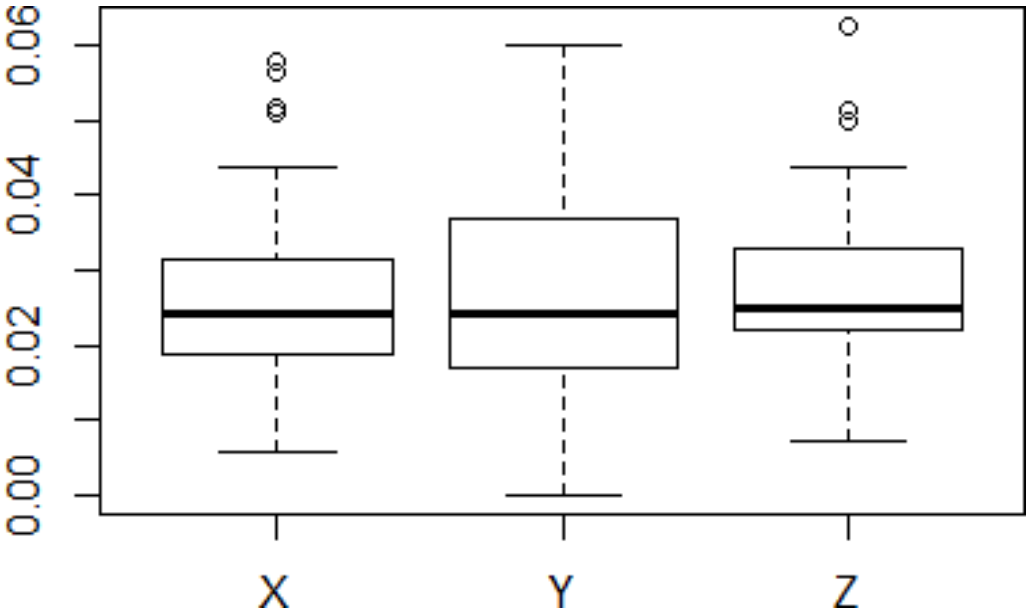

Figure 5. Average SD during rest for all individual subjects for all three axes.

For all subjects the average AI, maximum AI, the part active and the average intensity were calculated. The part active is the part of AI ≠ 0. The average intensity is the average AI of the AI ≠ 0. Using boxplots, Normal Q-Q plots and the Shapiro-Wilk tests, the data were checked for normality. Where necessary, the data were $\log_{10}$ transformed to yield normality, before group differences (lame vs. non-lame) and effects of treatment with Meloxicam in the lame piglets were assessed with independent samples and dependent Students *t*-tests respectively. The 95% confidence interval of the difference was used because this provides more information about the actual difference between groups (Cumming, 2008).

List of abbreviations

NSAIDS's	Nonsteroidal anti-inflammatory drugs
AI	Activity Intensity

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LR, EM, LV and FS contributed to the design of the study. The animal testing was performed by LR and LV. LR designed the R code to modify the accelerometer data. HV

gave advice for the statistical analysis, which was performed by LR. FS helped to draft the manuscript. All authors read and approved the final manuscript.

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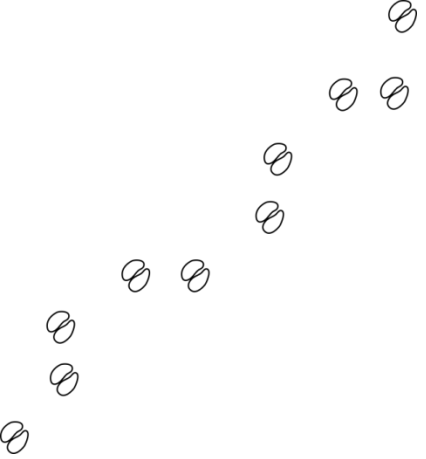

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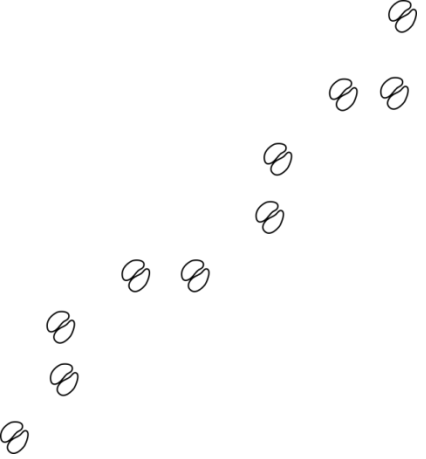


Samenvatting

Dutch summary

Het vermogen om zich voort te bewegen (locomotie) is van groot belang voor veel dieren, waaronder varkens. Locomotie kan bijvoorbeeld nodig zijn om aan roofdieren te ontkomen, om voedsel te vinden of om een partner te vinden. Voorbeweging komt tot stand door de geïntegreerde en gecoördineerde werking van het zenuwstelsel, spieren, botten, gewrichten, pezen en huid/hoorn. Als er een beschadiging optreedt in één of meer van deze weefsels kan er een mechanische of functionele belemmering plaatsvinden en kan een dier pijn ondervinden, met als gevolg is dat het dier kreupel gaat lopen.

Kreupelheid wordt gedefinieerd als "een onvermogen tot normale locomotie; een afwijking van de normale gang". Het is een veel voorkomend probleem bij varkens: prevalenties tot 19.7% bij vleesvarkens zijn gevonden. Kreupelheid bij varkens kan nadelige gevolgen hebben voor zowel de aangedane dieren als voor de veehouder.

Kreupele dieren kunnen een verminderd welzijn ondervinden. De belangrijkste oorzaak van verminderd welzijn is de pijn die gepaard gaat met kreupelheid. Daarnaast is het ook mogelijk dat dieren, doordat ze zich minder goed kunnen voortbewegen, bepaalde gedragingen niet meer (goed) kunnen uitvoeren. Zo kunnen ze bijvoorbeeld minder goed naar de voer- en drinkplaatsen en kunnen dus honger en dorst hebben. Ook kunnen ze zich minder goed staande houden in de sociale rangorde.

De gevolgen voor de veehouder bevinden zich vooral op het economische vlak. De kosten van behandeling van kreupele dieren, verminderde voedselopname (en daardoor verminderde groei) en de kosten van verhoogde sterfte of het vroegtijdig moeten euthanaseren van kreupele dieren kunnen allemaal bijdragen aan een verminderde opbrengst voor de veehouder.

Om de bovengenoemde negatieve gevolgen van kreupelheid te verminderen is het van belang om risicofactoren voor kreupelheid zoveel mogelijk te vermijden. Afhankelijk van de oorzaak van de kreupelheid zijn er verschillende risicofactoren aan te wijzen. In grote lijnen zijn deze op te delen in huisvesting, voeding en erfelijke factoren. Als er toch kreupelheid optreedt is het noodzakelijk om aangedane dieren te behandelen. Voor de behandeling van kreupelheid bij varkens zijn, afhankelijk van de oorzaak, twee hoofdgroepen van medicatie te onderscheiden: antibiotica en pijnstillers.

Antibiotica kunnen worden ingezet voor de behandeling van bacteriële oorzaken van kreupelheid, zoals een bacteriële gewrichtsontsteking. Ze zijn echter niet effectief tegen andere oorzaken en ze hebben geen pijnstillende werking. Pijnstillers zijn een andere, veel minder vaak toegepaste medicamenteuze behandelwijze van kreupelheid. Voor varkens zijn verschillende middelen geregistreerd, die allen onder de groep van de Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) vallen. Het bekendste voorbeeld van een NSAID is aspirine. NSAIDs hebben zowel een pijnstillende als een ontstekingsremmende

werking en kunnen bij uiteenlopende oorzaken van kreupelheid worden ingezet. Omdat ze een pijnstillende werking hebben, kunnen ze wellicht deze belangrijke component van welzijnsaantasting bij kreupele varkens verminderen.

Bovenstaande behandelingsopties zijn in sommige gevallen afdoende. Echter, in andere gevallen is behandeling onvoldoende of zelfs niet effectief. Er is daarom nog steeds een behoefte aan nieuwe, betere behandelingsopties en aan preventie. Om gericht onderzoek te kunnen doen naar het effect van dergelijke interventies is het belangrijk dat de **aanwezigheid** en de **mate** van kreupelheid op een betrouwbare en objectieve manier vastgesteld kunnen worden. Hier zijn verschillende mogelijkheden voor.

De meest eenvoudige, snelle en goedkope optie is het visueel ("op het oog") beoordelen van de locomotie van het dier. Deze methode vormt nog steeds de basis van het klinisch onderzoek door dierenartsen. Ook veehouders maken gebruik van visuele beoordeling van de locomotie van varkens om kreupele dieren te identificeren. Er zijn echter een aantal nadelen aan deze methode. Allereerst is het subjectief. Uit verschillende onderzoeken is gebleken dat het verwachtingspatroon van degene die de visuele inspectie uitvoert van invloed is op de uitkomst. Naast subjectiviteit is de matige herhaalbaarheid en overeenkomst tussen verschillende observeerders een belangrijk bezwaar. Bovendien is het moeilijk om in een groep varkens kreupele dieren te identificeren.

Om deze nadelen te ondervangen, zijn er verschillende methoden ontwikkeld om de locomotie van een dier op objectieve wijze te kunnen weergeven. Varkens zijn echter een lastige diersoort om op een dergelijke wijze locomotieonderzoek bij te doen. In tegenstelling tot bijvoorbeeld honden en paarden laten varkens zich moeilijk leiden aan een halsband of een tuigje. De snelheid waarmee ze lopen is daardoor amper te controleren. Helaas zijn de meeste parameters waar objectieve meetmethodes op gebaseerd zijn sterk afhankelijk van de snelheid van het dier.

Drukmatten leveren informatie over verschillende aspecten van de locomotie van varkens

In dit proefschrift is de bruikbaarheid van een drukmat om de aanwezigheid en de mate van kreupelheid vast te stellen geëvalueerd. Drukmatten bestaan uit een netwerk van een groot aantal druksensoren. Als een dier op de mat stapt, meet iedere sensor onder de poot afzonderlijk de druk. De informatie van de afzonderlijke sensoren wordt door software samengevoegd waardoor een pootafdruk met de verschillende drukken verkregen wordt. Een voordeel van de drukmat is dat er zowel informatie over de timing als de locatie van de pootafdrukken verkregen wordt. Hierdoor kunnen de afzonderlijke pootafdrukken van elkaar onderscheiden worden. Metingen hoeven daardoor niet

afgekeurd te worden als er meerdere poten tegelijk op de mat staan en als de mat lang genoeg is kunnen er meerdere pootafdrukken binnen 1 meting verzameld worden.

In **hoofdstuk 2** van dit proefschrift is gekeken naar vier verschillende parameters die door de drukmat gemeten kunnen worden: De maximale kracht die door een poot uitgeoefend wordt (Peak Vertical Force of PVF), de snelheid waarmee deze maximale kracht bereikt wordt (Load Rate of LR), de impuls die opgewekt wordt door de poot (Vertical Impulse of VI) en de maximale druk die onder de poot bereikt wordt (Peak Vertical Pressure of PVP). Deze vier parameters geven belangrijke informatie over de belasting van een individuele poot.

Belastingsparameters worden beinvloedt door verschillende variabelen

In **hoofdstuk 2** zijn de vier belastingsparameters wekelijks gemeten bij 10 gezonde biggen vanaf 5 weken leeftijd tot en met 15 weken leeftijd. Alle vier parameters verschilden sterk van week tot week en werden beïnvloed door de snelheid waarmee het dier over de mat liep. Ook maakte het uit of er naar een voor- of een achterbeen gekeken werd. Dit levert bezwaren op voor het gebruik van de drukmat bij kreupelheidsonderzoek. Het is dan immers niet duidelijk of variaties het gevolg zijn van snelheid, poot of meetmoment of van de kreupelheid.

Asymmetrie-indexen zijn een manier om de invloed van verstorende variabelen te verminderen

Om deze bezwaren te ondervangen werd er voor iedere parameter een zogenaamde Asymmetrie- Index (ASI) berekend, waarmee de linker voorpoot met de rechter voorpoot of de linker achterpoot met de rechter achterpoot vergeleken werd. Dit resulteerde in een waarde die theoretisch kon fluctueren tussen -200 (geen gewicht op de linker poot) en +200 (geen gewicht op de rechter poot). De verwachting was dat een gezond dier beide kanten evenredig zou belasten en de ASI in een gezond dier dus theoretisch 0 zou zijn. Door de parameters voor alle poten binnen 1 meting te verzamelen werd een deel van de variatie (bijvoorbeeld door verschillende snelheden) verwijderd. Dit resulteerde in ASI's die wel stabiel waren over de weken en die schommelden rond de 0.

Naast belastingsparameters registreert de drukmat nog meer gegevens, zoals de staplengten (de afstand die is afgelegd tussen een linker en een rechter pootje) en de standtijd (de tijd die een dier per stap op het pootje staat). Daar kunnen volgens hetzelfde principe als bij de belastingsparameters ook ASI's van berekend worden. In **appendix I** zijn ASI's van dergelijke parameters vergeleken tussen gezonde en kreupele varkens. Hoewel geconstateerd is dat deze significant van elkaar verschillen, waren er ook enkele

beperkingen, zoals de invloed van snelheid op de ASI van staptijd en de grote variatie binnen individuen.

ASI's van drukmat-parameters kunnen uiteraard ook voor andere diersoorten bepaald worden. In **appendix II** zijn ASI's berekend voor diverse drukmat-parameters van gezonde honden. In tegenstelling tot varkens, bleken de ASI's van honden wel onderhevig aan verschillende invloeden, zoals de kant waar de geleider liep, of er een bekende of onbekende geleider met de hond liep en in welke gewichtscategorie de hond zat. Het verschil tussen varkens en honden is dat varkens niet over de drukmat geleid werden maar er zelf overheen liepen. Daarnaast bestaat er een veel grotere variatie in lichaamsbouw en -grootte tussen honden onderling (vergelijk bijvoorbeeld een Teckel met een Rottweiler), waardoor ook meer variaties in ASI's kunnen ontstaan. Als er een drukmat gebruikt wordt om kreupelheid bij een hond te meten moet er dus met deze variabelen rekening gehouden worden.

Kreupele varkens belasten hun poten asymmetrischer dan gezonde varkens

Als een dier pijn heeft aan een poot zal het die poot proberen te ontzien. Dit resulteert in een asymmetrische belasting van de poten. Om te onderzoeken of kreupele dieren hun poten inderdaad minder symmetrisch belasten dan gezonde dieren, zijn in **hoofdstuk 3** de ASI's van 10 gezonde en 10 kreupele varkens met elkaar vergeleken. Kreupele dieren waren inderdaad significant minder symmetrisch. Omdat het dier zo min mogelijk gewicht op zijn aangedane poot wil zetten, zal het proberen dit gewicht naar de andere poten te verplaatsen. De dieren in dit experiment verplaatsten hun gewicht niet alleen naar de tegenoverliggende poot, maar ook naar de andere twee poten. Pijn in 1 poot heeft dus gevolgen voor alle andere poten.

In de kleine groep dieren in dit experiment was er voor ASI's van alle 4 parameters een duidelijke afkapwaarde te vinden waarmee onderscheid gemaakt kon worden tussen kreupele en gezonde dieren.

Pijnstilling kan de mate van kreupelheid verminderen

Naast de mogelijkheid om kreupelheid te identificeren met behulp van een drukplaat, is het ook van belang om een eventuele verbetering door bijvoorbeeld medicatie te kunnen meten.

Een mogelijke vorm van medicatie is pijnstilling. Kreupelheid kan het gevolg zijn van pijn. Een dier probeert de pijnlijke poot dan zo veel mogelijk te ontzien, wat kan resulteren in een asymmetrische belasting van de poten. Als kreupelheid (deels) veroorzaakt wordt door pijn zou een pijnstiller vermindering van de kreupelheid moeten geven.

In **hoofdstuk 4** is daarom onderzocht of het toedienen van een sterke pijnstiller (buprenorfine, een opiaat) resulteerde in een vermindering van de asymmetrie bij kreupele dieren. Dit bleek inderdaad het geval. Daarnaast werd een zogenaamde "open field test" uitgevoerd. In deze test werden de dieren individueel gedurende 10 minuten in een onbekende ruimte geplaatst en werd hun activiteit gescoord. Kreupele dieren waren actiever in de open field test nadat ze buprenorfine hadden gekregen dan dieren die een placebo hadden gekregen.

In **hoofdstuk 5** is de drukmat methode toegepast om het effect van een NSAID op een experimenteel opgewekte gewrichtsontsteking te beoordelen. De drukmat was daarbij onderdeel van een grotere proefopzet waarbij allerlei manieren om pijn bij dieren te meten werden gebruikt. Bij 20 dieren werd een stof (monoiodosodiumacetate of MIA) die het kraakbeen aantast in het linker polsgewricht gespoten, terwijl bij 20 andere dieren een controlemiddel dat geen effect heeft op het kraakbeen werd ingespoten. De injectie met MIA resulteerde in een subtiele kreupelheid die met het blote oog vaak niet waarneembaar was, maar die wel zichtbaar werd als een asymmetrische belasting op de drukmat. De toediening van een NSAID resulteerde niet in een vermindering van de asymmetrie.

De mate van kreupelheid kan niet één op één vertaald worden naar de mate van pijn

Uit bovenstaande paragraaf blijkt dat kreupelheid vaak samengaat met pijn. Toediening van een pijnstiller vermindert immers de ernst van de kreupelheid. Het is echter niet correct om de mate van kreupelheid één op één te vertalen naar de mate van pijn.

Als er sprake is van kreupelheid, hoeft er niet per definitie ook sprake te zijn van pijn. Kreupelheid (een afwijkende locomotie) kan, naast pijn, ook veroorzaakt worden door mechanische belemmeringen. Een voorbeeld hiervan is de uitgebreide botwoekeringen die op kunnen treden bij een chronische gewrichtsontsteking. De afwijkende locomotie is in dat geval deels het gevolg van pijn en deels het gevolg van mechanische beperkingen. Een andere oorzaak voor een afwijkende locomotie kan zijn dat de aansturing van de beweging niet goed verloopt. Dit treedt bijvoorbeeld op bij laesies van de grote hersenen. Deze laesies hoeven niet met pijn gepaard te gaan, en "kreupelheid" kan in die gevallen dus niet vertaald worden als "pijn", maar is het gevolg van een gestoorde aansturing van het bewegingsapparaat.

Het omgekeerde is ook mogelijk. Het is voor prooidieren zeer ongunstig om zwakte te tonen, want dat maakt ze een makkelijk slachtoffer voor roofdieren. Het is dus denkbaar dat een dier, ondanks dat het pijn ervaart, dit probeert te verbergen. De afwezigheid van kreupelheid betekent in een dergelijk geval niet per definitie dat het dier geen pijn ervaart.

Samengevat is de mate van kreupelheid dus slechts een afgeleide maat voor de mate van pijn. Bij gebrek aan methoden om pijn bij dieren direct te meten kan het echter wel een indicatie geven.

De mate van activiteit is een andere manier om kreupelheid te kwantificeren

Drukmatten zoals die in dit proefschrift gebruikt zijn kwantificeren één aspect van kreupelheid, namelijk een verminderde belasting van de aangedane poot. Echter, er zijn meerdere manieren waarop kreupele dieren zich aan trachten te passen. Zoals in **hoofdstuk 4** bijvoorbeeld is beschreven, is activiteit misschien een interessante parameter om het effect van interventies bij kreupele dieren te beoordelen. Het scoren van activiteit is echter zeer arbeidsintensief. Daarom is in **appendix III** een methode om automatisch activiteit te meten bij varkens in groepshuisvesting geëvalueerd. Om de activiteit te meten werden accelerometers (een soort stappentellers) gebruikt. Hoewel het praktisch uitvoerbaar bleek om de activiteit van varkens met deze accelerometers te meten, werden er geen verschillen in activiteit tussen kreupele en gezonde varkens gevonden.

Drukmatten zijn geschikt voor verschillende toepassingen

In dit proefschrift is een begin gemaakt met het onderzoeken van kreupelheid bij varkens met behulp van een drukmat. Er zijn in de toekomst verschillende toepassingen van dit meetinstrument denkbaar.

Allereerst biedt het een methode om de mate van kreupelheid objectief te meten en zij kan daarom gebruikt worden om allerlei interventies tegen kreupelheid te onderzoeken. Hierbij kan gedacht worden aan nieuwe medicijnen, maar ook bijvoorbeeld aan aanpassingen in de huisvesting van de dieren zoals een andere vloer.

Omdat ook drukprofielen binnen een klauw bekeken kunnen worden, kan er met behulp van de drukmat wellicht ook meer inzicht verkregen worden over de oorzaken en gevolgen van piekbelastingen op het hoorn van de klauwen. Klauwproblemen komen vooral bij zeugen zeer vaak voor. Uit onderzoek bij koeien is bekend dat gebieden waar veel druk op het hoorn staat kwetsbaarder zijn voor beschadigingen. Met behulp van een

drukmat zou een dergelijke relatie misschien ook voor het varken aangetoond kunnen worden.

Zoals al kort is besproken, zijn orthopedische aandoeningen niet de enige redenen waarom de locomotie aangetast kan worden. Verstoringen in de aansturing van de beweging vanuit het zenuwstelsel kunnen een grote invloed hebben op de voortbeweging. Dergelijke neurologische afwijkingen komen niet alleen bij varkens voor, maar ook bij mensen. Een drukmat zou gebruikt kunnen worden om een afwijkende locomotie ten gevolge van neurologische aandoeningen te kwantificeren.

Naast het gebruik van drukmatten om veterinaire vraagstukken op te lossen, zou het voor de humane geneeskunde ook interessante perspectieven kunnen bieden. Varkens worden in toenemende mate gebruikt als modeldier voor de mens, onder andere in onderzoek naar traumatisch hersenletsel. Het gebruik van een drukmat geeft onderzoekers een objectieve manier om de ernst van de gangafwijking in een diermodel te kwantificeren.

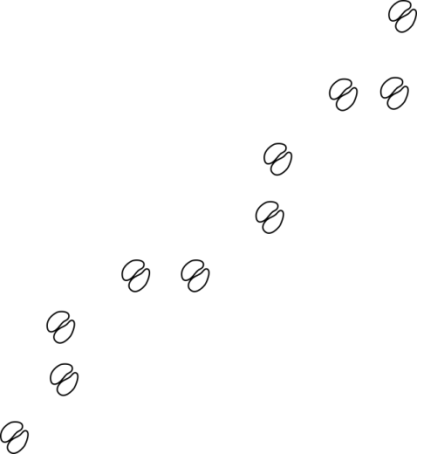
Naast de verschillende toepassingen voor onderzoeksdoeleinden, zouden drukmatten ook gebruikt kunnen worden om in varkensstallen, onder praktijkomstandigheden kreupele dieren te detecteren. Te denken valt bijvoorbeeld aan het inbouwen van een drukmat op het pad naar een voerstation. Hoewel deze toepassing in theorie mogelijk is, zijn er een aantal praktische bezwaren. Allereerst is de huidige software gemaakt voor mensen en kan niet automatisch onderscheid maken tussen afdrukken van de vier poten van varkens. Toewijzing moet vooralsnog met de hand gebeuren en dit kost veel tijd. Ook moet er een algoritme ontwikkeld worden dat automatisch de randvoorwaarden voor een goede meting controleert (liep het dier recht, op constante snelheid, keek het niet opzij?). Daarnaast hebben lang niet alle stallen voerstations en zal er in veel gevallen ruimte gemaakt moeten worden om de drukmat neer te leggen. Al met al zijn er tot nu toe nog te veel bezwaren om drukmatten op een dergelijke wijze in de praktijk te gaan gebruiken.

Conclusie

Drukmatten zijn geschikt om informatie over verschillende aspecten van de locomotie van varkens te verzamelen. In dit proefschrift is vooral gekeken naar belastingsparameters. Deze belastingsparameters worden beïnvloed door verschillende variabelen, bijvoorbeeld loopsnelheid. Omdat het bij varkens niet mogelijk is om deze variabelen constant te houden tussen de verschillende metingen, is er in dit proefschrift voor gekozen om de verschillende poten binnen een meting met elkaar te vergelijken. Deze vergelijking resulteert in een asymmetrie-index en is een bruikbare maat voor de ernst van kreupelheid bij een varken.

Locomotie-analyse met behulp van een drukmat is, in tegenstelling tot visuele beoordeling van de locomotie, een objectieve techniek. Daarnaast zijn drukmatten beter in staat om subtiele verschillen, zoals het effect van een pijnstiller, te detecteren dan dat visuele beoordeling dat kan.

Drukmatten kunnen gebruikt worden voor verschillende onderzoekstoepassingen, zowel voor het oplossen van veterinaire vraagstukken als in dierexperimenteel onderzoek ten behoeve van de humane geneeskunde. Het gebruik van drukmatten in de varkenshouderij om onder praktijkomstandigheden kreupelheid op te sporen stuit vooralsnog echter nog op te veel praktische bezwaren.



Dankwoord

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toch maar mooi gered en dat was te danken aan jouw humor, doorzettingsvermogen en eeuwige goede humeur.

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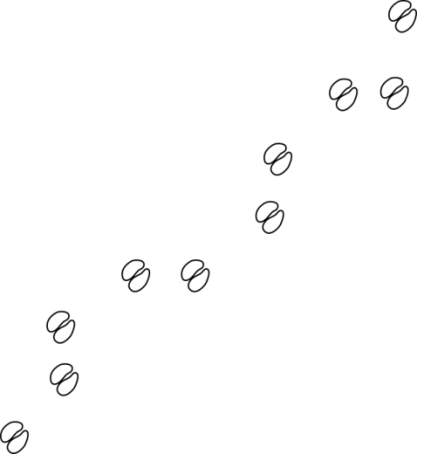

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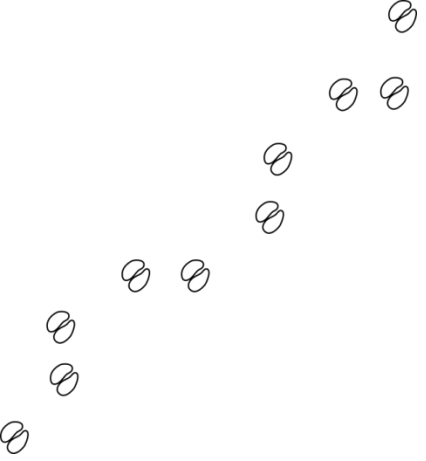

Curriculum Vitae

Ellen Meijer (Neede, 15 september 1980) graduated from “Het Assink College” (VWO) in 1998. After one year of studying Animal Sciences at Wageningen University and a year working at a large horse training facility in Brentwood, New York she enrolled in the Veterinary Medicine program at Utrecht University in 2000. She graduated in 2006 from the Companion Animal track. During the last year of her study she won both the Royal Canin Student Award for clinical cases and the Boehringer Ingelheim Poster Award at the Voorjaarsdagen veterinary conference.

After graduating she worked in a companion animal practice in Rotterdam (2007/2008) and a mixed practice in Noord Holland (2008). She was a substitute teacher for both the “laboratory animal” course and the “first aid for animals” course at the Groenhorst College in Barneveld from September 2008 to December 2008.

In December 2008 she started working at the Clinical Pathophysiology Group of the Department of Farm Animal Health of the faculty of Veterinary Medicine at Utrecht University. After working as a junior teacher for three years, she started her PhD on the quantification of lameness in pigs in October 2011 at the Animal Behaviour and Welfare group (at that time known as the Emotion and Cognition group), which is also part of the Department of Farm Animal Health. As of December 2015 she has continued her career at the Animal Behaviour and Welfare Group, focusing on both research and teaching.