




Two-dimensional and three-dimensional techniques for determining the kinematic patterns for hindlimb obstacle avoidance during sheep locomotion

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ABSTRACT: Analysis of locomotion is often used as a measure for impairment and recovery following experimental peripheral nerve injury. Compared to rodents, sheep offer several advantages for studying peripheral nerve regeneration. In the present study, we compared for the first time, two-dimensional (2D) and three-dimensional (3D) hindlimb kinematics during obstacle avoidance in the ovine model. This study obtained kinematic data to serve as a template for an objective assessment of the ankle joint motion in future studies of common peroneal nerve (CP) injury and repair in the ovine model. The strategy used by the sheep to bring the hindlimb over a moderately high obstacle, set to 10% of its hindlimb length, was pronounced knee, ankle and metatarsophalangeal flexion when approaching and clearing the obstacle. Despite the overall time course kinematic patterns about the hip, knee, ankle, and metatarsophalangeal were identical, we found significant differences between values of the 2D and 3D joint angular motion. Our results showed that the most apparent changes that occurred during the gait cycle were for the ankle (2D-measured STANCEmax: 157±2.4 degrees vs. 3D-measured STANCEmax: 151±1.2 degrees; $P < .05$) and metatarsophalangeal joints (2D-measured STANCEmin: 151±2.2 degrees vs. 3D-measured STANCEmin: 162 ± 2.2 degrees; $P < .01$ and 2D-measured TO: 163±4.9 degrees vs. 3D-measured TO: 177±1.4 degrees; $P < .05$), whereas the hip and knee joints were much less affected. Data and techniques described here are useful for an objective assessment of altered gait after CP injury and repair in an ovine model.

Key words: quadruped, 2D and 3D gait analysis, biomechanics, joint angle, peripheral nerve injury.

Técnicas bidimensionais e tridimensionais para determinar os padrões cinemáticos durante a ultrapassagem de obstáculos pelos membros pélvicos durante a locomoção de ovinos

RESUMO: A análise da locomoção é frequentemente usada como uma medida para avaliar a disfunção e sua recuperação após lesão nervosa periférica experimental. Quando comparadas com os roedores, as ovelhas oferecem várias características atrativas como modelo experimental para o estudo da regeneração nervosa periférica. Não existem estudos acerca dos resultados da locomoção após lesão e reparação do nervo periférico no modelo ovino. No presente estudo, realizámos e comparámos a cinemática bidimensional (2D) e, pela primeira vez, tridimensional (3D) do membro pélvico durante a ultrapassagem de obstáculos no modelo ovino. Este estudo teve como objetivo obter dados cinemáticos para servir de modelo para uma avaliação objetiva do movimento articular do tornozelo em estudos futuros de lesão e reparação do nervo fibular comum (FC) no modelo ovino. A estratégia usada pelas ovelhas para elevar o membro pélvico sobre um obstáculo com uma altura moderada, fixado em 10% do seu comprimento, caracteriza-se por uma flexão pronunciada do joelho, tornozelo e metatarso-falangeana ao se aproximar e ultrapassar o obstáculo. Apesar dos padrões cinemáticos do quadril, joelho, tornozelo e metatarso-falangeano terem sido idênticos, foram encontradas diferenças significativas entre os valores do movimento angular das articulações em 2D e 3D. Os nossos resultados mostram que as mudanças mais aparentes que ocorreram durante o ciclo da marcha foram nas articulações do tornozelo (em 2D STANCEmax: 157±2.4 graus vs. em 3D STANCEmax: 151±1.2 graus; $P < .05$) e metatarso-falangeana (em 2D STANCEmin: 151±2.2 graus vs. em 3D STANCEmin: 162 ± 2.2 graus; $P < .01$ e em 2D TO: 163±4.9 graus vs. em 3D TO: 177±1.4 graus; $P < .05$), enquanto as articulações do quadril e do joelho foram muito menos afetadas. É provável que os dados e técnicas descritas aqui sejam úteis para uma avaliação objetiva das alterações na marcha após lesão e reparação do PC no modelo ovino.

Palavras-chave: quadrúpede, análise de marcha em 2D e 3D, biomecânica, ângulo articular, lesão do nervo periférico.

INTRODUCTION

Because of their spread distribution throughout the body, peripheral nerves are particularly subject to traumatic injuries in animals and humans (MOHANNA et al., 2003; FORTERRE et al., 2007; SIEMIONOW & BRZEZICKI, 2009). Given that most experimental studies on microsurgical repair have been performed on major hindlimb nerves, animal gait analysis is often used as a measure for impairment and recovery. Over the years, several gait kinematics have been developed to analyse locomotor deficits following injury in rodents, particularly in the rat sciatic nerve model (FILIFE ET AL., 2006; COSTA ET AL., 2009; VELA et al., 2020).

Sheep offer several attractive features as model organisms for studying peripheral nerve injury and repair. First, nerves in sheep and in humans have similar size and regeneration behaviour (GLASBY et al., 1993; FULLARTON et al., 2001). Second, they are attractive models for biomedical and basic biological research because of availability and they can be kept in natural groups with very basic housing demands. Third, they are placid animals and can be readily trained for gait analysis. Fourth, the larger size of the sheep facilitates more precise and potentially more accurate measurement of joint kinematics. Fifth, due to its relatively large size, sheep provide sufficient nerve tissue samples for other histological and molecular biological analysis. Finally, in comparison to other large mammals like primates, dogs and cats, sheep are better accepted by society as a research animal and also represent a less expensive alternative (OZTURK et al., 2016).

Animal studies using the sheep as kinematic model are scarce in the literature and only in the field of orthopaedics and spinal cord injury research (BEVERIDGE et al., 2011; FRANK et al., 2012; VALENTIN et al., 2014; WILSON et al., 2017). Recently, our lab used a two-dimensional (2D) model to analyse the hindlimb kinematics in clinically healthy sheep during overground and obstacle avoidance during locomotion (COSTA et al., 2018). We were able to quantify the compensatory strategies that take place at each joint in response to obstacle avoidance during locomotion in the ovine model. A considerable advantage of using a 2D kinematic approach is the simplicity of the procedure, which requires only one camera to record the movement. However, it seems reasonable to assume that the hip, knee, ankle and metatarsophalangeal joints may translate out of a parasagittal plane under some conditions, such as obstacle avoidance.

In the light of reported literature, no comparisons have been made between 2D and three-dimensional (3D) hindlimb kinematics during obstacle avoidance in the ovine model. The purpose of this study was to compare hindlimb kinematics using 2D and 3D approaches in clinically normal sheep during obstacle avoidance and create a template for an objective assessment of the ankle joint motion following common peroneal nerve (CP) injury and repair in the ovine model.

MATERIALS AND METHODS

Ovine model

This investigation was approved by the Institutional Animal Care and Use Committee of the University of Trás-os-Montes e Alto Douro (IACUC Approval No. 6/2015). All procedures were performed with the approval of the Portuguese Veterinary Authorities, in accordance with the EU Directive 2010/63/EU for animal experiments. Four female Portuguese Churra-da-Terra Quente breed experimental sheep (age: around 2 years; weight: around 40 kg) belonging to a breeding colony based at University of Trás-os-Montes e Alto Douro were used in this study. Upon delivery, all animals were examined by a veterinarian and deemed non-pregnant and free of disease. The sheep were judged to be healthy on account of results of complete physical, orthopaedic and neurologic examinations. The animals were fed with a diet of hay supplemented with concentrate according to their requirements and had free access to fresh water. The present experimental design did not induce any pain or discomfort to the included ovine, and by the end of the studied timepoint, all the animals returned to their normal activities without any disturbance nor pathology.

Training procedure

The general procedure for training was similar to that in previous research (COSTA et al., 2018). Two weeks before the collection of kinematic data, sheep were trained daily for 30 minutes to walk overground at a natural speed on a 2 m by 0.6 m walkway and to step over a rectangular obstacle (6 cm high × 6 cm wide).

Kinematic recording

Hemispherical markers with a diameter of 2 cm were placed on the skin over six anatomic landmarks on the lateral side of the left hindlimb: the dorsal point of the iliac crest, the greater trochanter, the estimated joint centre of the knee, the lateral

malleolus, the distal end of the metatarsal bone and distal end of the middle phalanx, as in the previous study (COSTA et al., 2018).

Three CMOS cameras (PhotonFocus MV-D640C, Lachen, Switzerland) were strategically placed around the left hindlimb to minimize marker occlusion, maximize resolution and to improve the accuracy of the 3D reconstruction process. The camera in the middle, which was placed perpendicular to the direction of the movement, was used for 2D analysis. Kinematic data were collected at a sampling rate of 144 Hz. The cameras' field of view was calibrated to cover 2 meters in length of the walkway and allowed the recording of a complete gait cycle (Figure 1). The images were acquired using the software Video Savant 4 (IO Industries Inc, Ontario, Canada). The color image had a resolution of 640×480 pixels. We designed a new calibration object, which allowed us to calibrate all three cameras at the same time. The use of the calibration object ensured that the projection matrixes were all related to the same coordinate system. This allowed all points to be used to compute the kinematic parameters without any need for their transformation. The 3D coordinates obtained were compared with 3D coordinates measured in the calibration object, and for each point, a position

error was calculated. This error was computed as the Euclidean distance between the measured and the reconstructed 3D coordinates. We obtained a mean absolute position error of 3.41 ± 2.9 mm. The camera calibration and the 3D reconstruction process were similar to a previously described procedure (DIOGO et al., 2017).

During each trial, sheep walked at a natural speed over the walkway, led by a handler using a halter. The present research limited walking velocity to between 1.1-1.5 m/s (COSTA et al., 2018). A total of ten trials for each sheep were analysed. Therefore, to ensure a reliable comparison between the 2D and 3D sagittal plane kinematics of the hindlimb joints, a total of 40 gait cycles were obtained during obstacle avoidance from 4 clinically healthy sheep.

Joint flexion-extension angles were measured at the flexor side for hip, knee, ankle and metatarsophalangeal (MTP) joints. For the 2D approach, the knee position was computed indirectly by superimposing two circles (centred on hip and ankle pivots) with a radius of the femur and tibia length, respectively. The knee position was determined as the intersection of the two circles. These bone lengths were obtained from radiographic images. The animals were anesthetized and radiographed in dorsal recumbency

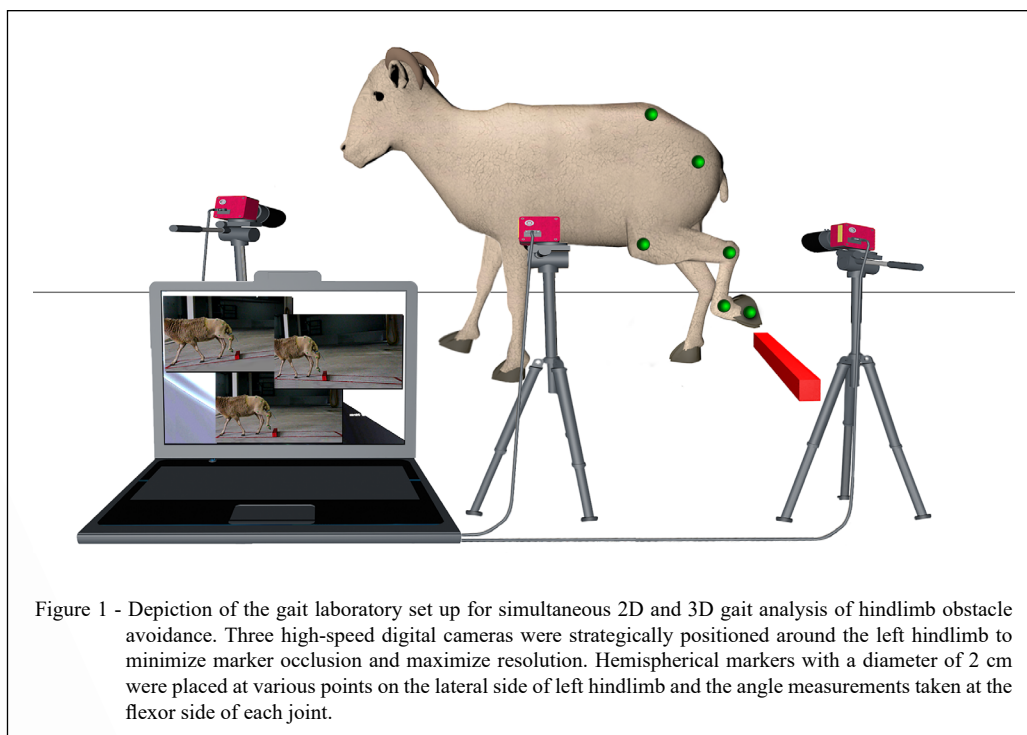


Figure 1 - Depiction of the gait laboratory set up for simultaneous 2D and 3D gait analysis of hindlimb obstacle avoidance. Three high-speed digital cameras were strategically positioned around the left hindlimb to minimize marker occlusion and maximize resolution. Hemispherical markers with a diameter of 2 cm were placed at various points on the lateral side of left hindlimb and the angle measurements taken at the flexor side of each joint.

with the hindlimbs outstretched using a radiography machine (Philips Optimus 80, Hamburg, Germany). Radiographs were taken with a setting of 60 kVp and 15 mAs. The images were obtained using Kodak film and were processed routinely using an automated radiograph developer. The measurements were made to the nearest millimetre. For the 3D biomechanical model, we applied a slightly different approach. The two circles were replaced by two spheres centred on the greater trochanter and the lateral malleolus with a radius equal to the length of the femur and tibia and segments, respectively. We presumed that the true position of the knee marker would lie on the plane defined by the greater trochanter, the knee and the lateral malleolus markers. From the intersection of this plane with the circumference resulting from the intersection of the two spheres, we obtained two points. We choose the one that was consistent with the direction of movement, as previously described (Figure 2) (COUTO et al., 2008).

Numerical analysis

The general procedure for numerical analysis was similar to our recent paper (COSTA et al., 2018). The marker-based angular kinematic curves were filtered using a fourth-order Butterworth filter (cut-off frequency at 10Hz). For each stride, the duration of the stance and swing phases was normalized. Cubic spline interpolation was applied to the original data regarding the angular position of the pelvis, hip, knee, ankle, and MTP joints to obtain 101 samples per gait cycle regardless of their duration. We recorded the maximal and minimal joint flexion-extension angles during both the stance and swing phases of the gait, as well as the angles at the point of initial hoof-ground contact (IC, start of the stance phase) and immediately when the hoof is lifted from the ground (TO, start of the swing phase). This numerical analyse was performed using Matlab computational software (The MathWorks Inc., Natick, MA, USA).

In addition, the following gait parameters were included: gait cycle duration, stance duration, swing duration, stride length and the maximal vertical displacement (MVD). Stride length was defined by the distance between the middle phalanx markers of the hindlimb in two consecutive steps. Maximal vertical displacement was quantified by measuring the maximum height reached by the middle phalanx marker when stepping over the obstacle (Figure 3).

Statistical analysis

Differences in joint kinematics data collected in 2D and 3D were tested using paired

Student's t-test. Mean \pm standard deviation (SD) values for all the measured variables are reported. The two-way mixed model intra-class correlation coefficient (ICC) for absolute agreement was calculated as a preliminary measurement of intra-trial, inter-step reliability of joint kinematics data. The statistical significance was set at the level of $P < 0.05$. All statistical tests were performed using IBM SPSS Statistics V22.0 software.

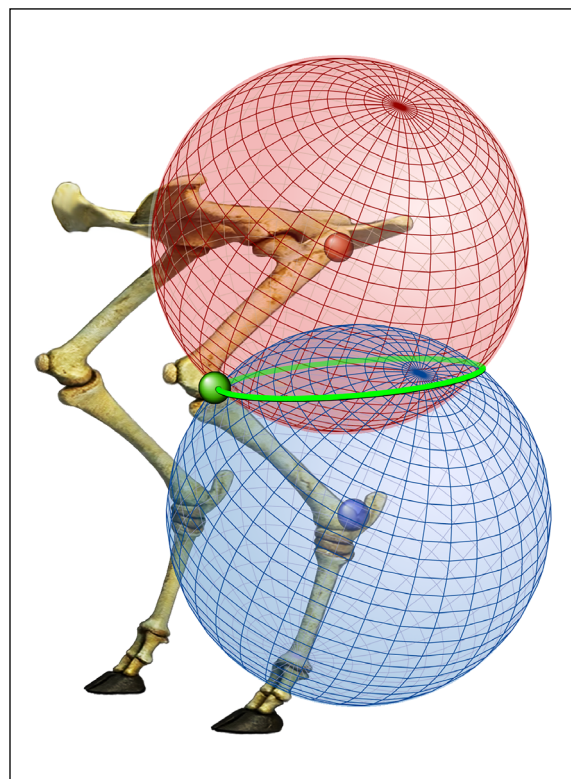
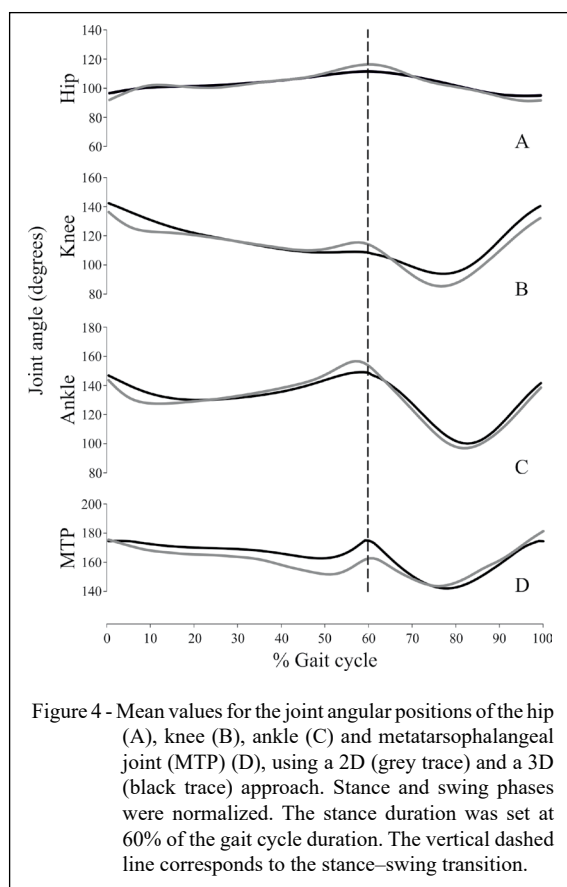
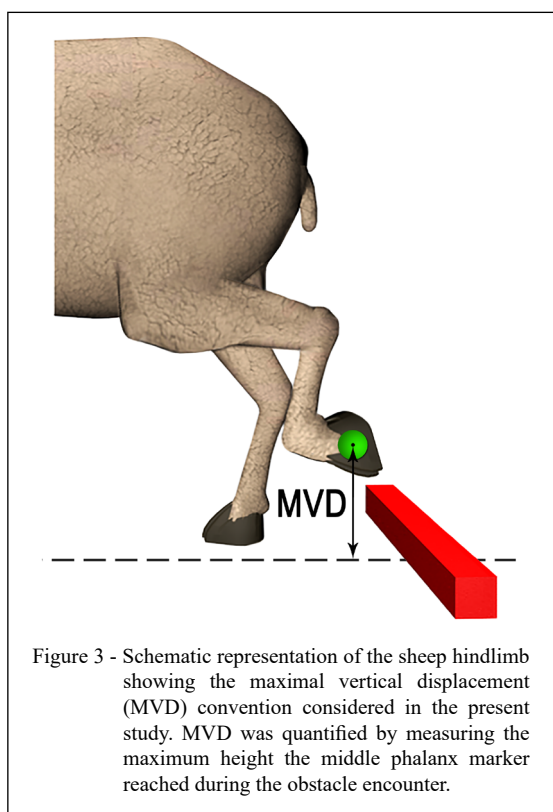


Figure 2 -Three-dimensional estimation of the knee marker. The knee position was computed indirectly by superimposing two spheres centred on the 3D positions of the greater trochanter (red) and the lateral malleolus (blue) and with a radius equal to the length of the femur and tibia segments, respectively. It was presumed that the true position of the knee marker would lie on the plane defined by the greater trochanter, the knee and the lateral malleolus markers. From the intersection of this plane with the circumference (green) resulting from the intersection of the two spheres, we obtained two points. We choose the one that was consistent with the direction of movement. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)



RESULTS

Spatio-temporal measurements

Mean (\pm S.D.) values obtained for the whole group were 1.36 ± 0.10 m/s for walking speed, 693 ± 45 ms for gait cycle duration, 416 ± 57 ms for stance duration, 277 ± 25 ms for swing duration, 94 ± 5.0 cm for stride length and 10.1 ± 0.8 cm for MVD during obstacle avoidance.

Angular kinematic parameters

The general shapes of the 2D and 3D joint kinematic patterns for the hip, knee, ankle and MTP were very similar (Figure 4). The average toe-off (TO) time was calculated to be 60% into the step cycle. Despite similar 2D and 3D overall joint patterns, for MTP, minimal angle during stance (STANCE_{min}) ($p < 0.01$; Table 1) and the angle at TO were both reported to be significantly increased (i.e., less flexion) when measured in 3D ($p < 0.05$; Table 1A). For the ankle, the maximal angle during stance (STANCE_{max}) was decreased when recorded in 3D ($p < 0.05$; Table 1). No other differences in hindlimb kinematics between the two setups existed.

Intra-trial ICC values for joint kinematics data are displayed in table 2. As could be expected, ICC values ranged between good (values between 0.75 and 0.90) to excellent (values above 0.90) for most joint kinematics data. However, a few measurements displayed low reliability. The latter mainly pertained to joint angles measured from the distal joints of the hindlimbs, namely the MTP and the ankle (Table 2).

DISCUSSION

The purpose of this study was to compare joint hindlimb kinematics in normal sheep during obstacle avoidance calculated by a 2D and a 3D method. The 2D and 3D calculations were based on kinematic data from the same experiments to ensure that the comparison was reliable. These assessments provide novel information on how each joint contributes to obstacle avoidance during overground locomotion in the ovine model, while also demonstrating the good to excellent reliability of amplitudes and timing of

Table 1 - Joint angle data.

	-----MTP (degrees)-----		-----Ankle (degrees)-----		-----Knee (degrees)-----		-----Hip (degrees)-----	
	2D	3D	2D	3D	2D	3D	2D	3D
IC	176 ± 3.3	175 ± 2.7	145 ± 7.8	147 ± 4.8	137 ± 12.2	143 ± 6.7	93 ± 8.6	96 ± 3.4
STANCEmin	151 ± 2.2	162 ± 2.2**	127 ± 4.9	130 ± 3.1	110 ± 9.2	107 ± 4.9	93 ± 8.9	96 ± 3.4
STANCEmax	177 ± 3.0	178 ± 2.0	157 ± 2.4	151 ± 1.2*	137 ± 12.2	143 ± 6.2	114 ± 9.7	111 ± 7.4
TO	163 ± 4.9	177 ± 1.4*	151 ± 3.7	149 ± 1.7	114 ± 6.3	109 ± 6.7	114 ± 10.2	111 ± 7.4
SWINGmin	138 ± 16	141 ± 2.6	96 ± 4.6	101 ± 4.4	86 ± 9.7	94 ± 4.4	91 ± 6.9	94 ± 4.4
SWINGmax	182 ± 2.3	179 ± 0.3	151 ± 3.7	150 ± 1.1	134 ± 11.1	141 ± 6.4	114 ± 10.4	111 ± 7.2

IC: initial contact; STANCEmin: minimal angle during stance; STANCEmax: maximal angle during stance; TO: toe-off; SWINGmin: minimal angle during swing; SWINGmax: maximal angle during swing; MTP: Metatarsophalangeal; 2D: two-dimensional; 3D: three-dimensional.

Significantly different from 2D; * $p < .05$; ** $p < .01$.

joint trajectories when recorded in a single session. In order to reduce the flocking behavior, 2 weeks before data collection, sheep were trained to walk over the walkway at a comfortable speed, led by the same handler using a halter. This improved the accuracy of the kinematic gait parameters and minimized the number of animals used. (HARGREAVES et al., 1997).

In future studies of surgical transection and repair of peripheral nerves, we will selectively choose the common peroneal nerve (CP) in the ovine model. Sheep CP is a medium nerve with a diameter similar to frequently injured human nerves, allowing for much longer nerve gaps when compared to rats. The CP innervates only muscles of dorsiflexion, namely,

the cranial tibial and peroneus tertius, and no antagonistic muscle groups. An important consequence of an experimental CP injury is the loss of ankle dorsiflexor activity. Therefore, this new biomechanical model will allow an objective assessment of ankle locomotor mechanics and the compensatory strategies for future nerve regeneration studies.

In this model, a 6 cm high obstacle was placed in the middle of the walkway and all animals showed an adequate ankle dorsiflexion, allowing the necessary hoof-avoidance, while maintaining forward progression. Choosing the correct height for the obstacle, reflecting 10% of the animals' hindlimb length, was essential for a safe and successful

Table 2 - Intra-class correlation values for inter-step joint angle data.

	-----MTP (degrees)-----		-----Ankle (degrees)-----		-----Knee (degrees)-----		-----Hip (degrees)-----	
	2D	3D	2D	3D	2D	3D	2D	3D
IC	0.447	0.840	0.976	0.838	0.907	0.936	0.989	0.977
STANCEmin	0.703	0.691	0.961	0.827	0.922	0.948	0.991	0.977
STANCEmax	0.561	0.900	0.853	0.594	0.907	0.921	0.995	0.960
TO	0.949	0.352	0.891	0.187	0.926	0.949	0.993	0.956
SWINGmin	0.968	-0.176	0.877	0.807	0.808	0.603	0.986	0.966
SWINGmax	0.127	0.503	0.891	0.306	0.901	0.959	0.992	0.954

IC: initial contact; STANCEmin: minimal angle during stance; STANCEmax: maximal angle during stance; TO: toe-off; SWINGmin: minimal angle during swing; SWINGmax: maximal angle during swing; MTP: Metatarsophalangeal; 2D: two-dimensional; 3D: three-dimensional.

obstacle-crossing, as previously described (NIANG et al., 2005).

The results of the 2D and 3D kinematic data showed that the general shapes of joint angular motion were very similar and only few statistically significant differences in angle values at selected points of the gait cycle existed between the two set-ups. Differences between 2D and 3D joint kinematics were only reported at the MTP and the ankle, whereas the magnitudes of the hip and knee flexion-extension angles were the same, regardless of being recorded in 2D or 3D. This finding could be the result of transverse plane motion of the most distal joints, which could not be accounted for in the 2D video assessment. (SCHURR et al., 2017). Although, our results support the use of the simpler 2D method, as most of the joint angles were similarly measured in 2D and 3D, there were some consistent differences between the two motion analysis methods. Previous studies also report differences in rat toe and ankle flexion-extension joint position during level walking between 2D and 3D biomechanical analysis, which agrees with the present results (JOÃO et al., 2010). The fact that 3D-measured MTP and ankle angles might subtly but consistently differ from 2D could be relevant for applications in peripheral nerve research employing the sheep model and the CP nerve, as injury of this nerve specifically affects innervation of the skeletal muscles crossing those joints. Further studies comparing 2D and 3D sheep hindlimb motion using animals recovering from CP injury are warranted.

The high degree of stability of hindlimb flexion-extension in our biomechanical model was also demonstrated by the good to excellent inter-step reliability observed for a large majority of gait measurements in this study. However, poor reliability was reported for a limited number of MTP and ankle angle measurements. At this point, it is uncertain whether such low ICC values reflect normal variation in joint kinematics during obstacle avoidance or is related to study design, in particular the limited number of animals.

It is important to acknowledge several limitations of the data collected for this study. First, incorrect placement of markers with respect to the anatomy is a known source of error in the joint kinematic outputs (MCGINLEY et al., 2009). However, since one investigator applied the markers over palpable bony landmarks on all study animals, it is expected that these errors would be minimized and be similar across all trials.

Second, displacement of the skin surface relative to knee joint is the principle source of error

when estimating hindlimb joint kinematics in quadrupeds, due to a more extensive skin attachment from the proximal hindlimb to the lateral torso when compared to humans (MUIR & WEBB, 2000). The 3D biomechanical knee model was made possible by using radiography and applying the anatomical findings to biomechanical principles. The knee joint should operate aligned with the plane defined by the greater trochanter, the knee and the lateral malleolus. We may consider the knee as a hinge joint with one degree of freedom, moving from extension into full flexion along the sagittal plane (EKEBERG & PEARSON, 2005). Third, we did not collect electromyographic recordings in this study. As a result, we cannot compare the observed changes in joint motion with the recruitment of the different muscles of the hindlimb.

CONCLUSION

In summary, the present study established fundamental 3D kinematic characteristics of ankle joint motion and the compensatory strategies that take place at each joint of the hindlimb in response to obstacle avoidance in the ovine model. The kinematic results presented here should provide direction for future studies following CP injury and repair. It is also expected that this *in vivo* 3D hindlimb model will prove useful to analyse future biomechanical studies, involving other peripheral nerves of the lumbosacral plexus. We also anticipate that this model will serve as an effective tool to compare gait pathologic conditions and improve our knowledge on kinematic features associated with other experimental neurological conditions, including junctionopathies.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This investigation was approved by the Institutional Animal Care and Use Committee of the University of Trás-

os-Montes e Alto Douro (IACUC Approval No. 6/2015). All procedures were performed with the approval of the Portuguese Veterinary Authorities, in accordance with the EU Directive 2010/63/EU for animal experiments.

DECLARATION OF CONFLICTS OF INTERESTS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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