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# Validation of 2 noninvasive, markerless reconstruction techniques in biplane high-speed fluoroscopy for 3-dimensional research of bovine distal limb kinematics

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## ABSTRACT

Lameness severely impairs cattle's locomotion, and it is among the most important threats to animal welfare, performance, and productivity in the modern dairy industry. However, insight into the pathological alterations of claw biomechanics leading to lameness and an understanding of the biomechanics behind development of claw lesions causing lameness are limited. Biplane high-speed fluoroscopic kinematography is a new approach for the analysis of skeletal motion. Biplane high-speed videos in combination with bone scans can be used for 3-dimensional (3D) animations of bones moving in 3D space. The gold standard, marker-based animation, requires implantation of radio-opaque markers into bones, which impairs the practicability for lameness research in live animals. Therefore, the purpose of this study was to evaluate the comparative accuracy of 2 noninvasive, markerless animation techniques (semi-automatic and manual) in 3D animation of the bovine distal limb. Tantalum markers were implanted into each of the distal, middle, and proximal phalanges of 5 isolated bovine distal forelimbs, and biplane high-speed x-ray videos of each limb were recorded to capture the simulation of one step. The limbs were scanned by computed tomography to create bone models of the 6 digital bones, and 3D animation of the bones' movements were subsequently reconstructed using the marker-based, the semi-automatic, and the manual animation techniques. Manual animation translational bias and precision varied from 0.63 ± 0.26 mm to 0.80 ± 0.49 mm, and rotational bias and precision ranged from 2.41 ± 1.43° to 6.75 ± 4.67°. Semi-automatic translational values for bias and precision ranged from 1.26 ± 1.28 mm to 2.75 ± 2.17 mm, and rotational values varied from 3.81 ± 2.78° to 11.7 ± 8.11°. In our study, we demonstrated the successful application of biplane high-speed fluoroscopic kinematography to gait analysis of bovine distal limb. Using the manual animation technique, kinematics can be measured with sub-millimeter accuracy without the need for invasive marker implantation.
**Key words:** dairy cow, locomotion, 3D animation, bone model

## INTRODUCTION

Lameness is one of the biggest threats to the dairy industry, with substantial negative effects on well-being, performance, and productivity of affected dairy cows worldwide (Warnick et al., 2001; Green et al., 2002; Bicalho et al., 2008).

Scientific analysis of the underlying causes, particularly a biomechanical understanding of claw–floor interactions, is key for successful reduction of claw tissue damage and the detection and prevention of lameness (Main et al., 2012). So far, knowledge of bovine kinematics and locomotion is based on high-speed cinematography (Meyer et al., 2007; Schmid et al., 2009; Blackie et al., 2013) or traditional radiographs (Ehlert, 2006; El Shafaey et al., 2013). But these analyses have difficulties characterizing motion of a joint that is capable of 6 degrees of freedom of movement.

Biplane high-speed fluoroscopic kinematography (**HFK**) is a new approach to measuring bone alignment and its changes during movement with high accuracy (Brainerd et al., 2010). This technique allows for analysis of 3-dimensional (**3D**) bone motion, including visualization of the distal phalanx through the surrounding horn capsule (Panagiotopoulou et al., 2016). Two x-ray generators in combination with 2 fluoroscopic image intensifiers capture high-speed video sequences (Figure 1). These sequences serve as templates for 3D bone animations of bone reconstructions and enable direct and precise measurements of bone elements in live animals during locomotion (Tashman and Anderst, 2003). So far, 3D animations have been used for motion analysis in human research (Bey et al., 2008; McDonald et al., 2010; Anderst et al., 2011; Baka et al., 2012) and

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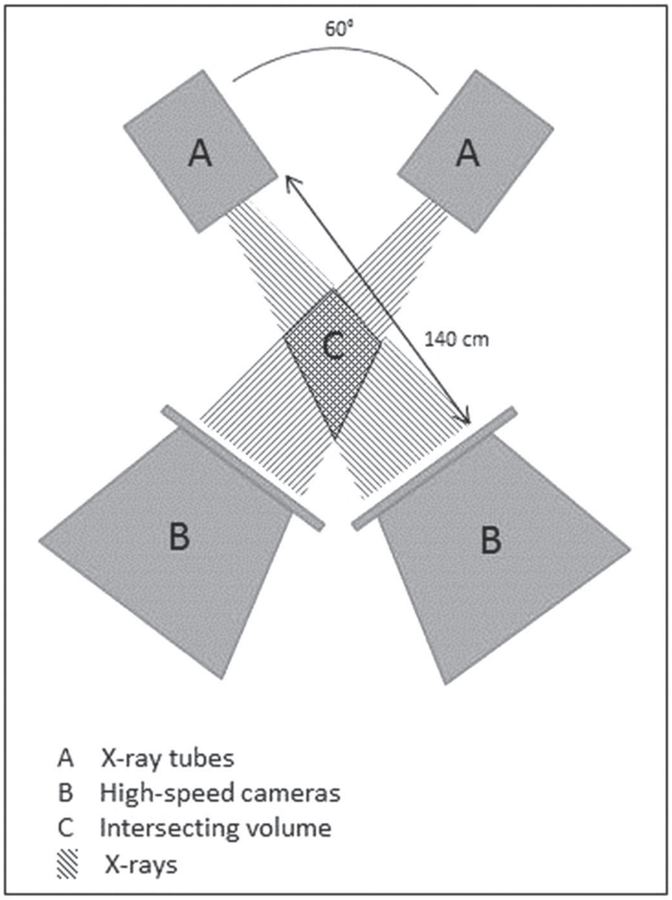


**Figure 1**. Setup of the biplane high-speed radiographic imaging system. The system operated with an interbeam angle of 60° and an x-ray source-to-image distance of 140 cm. The setup was selected with regard to applicability to live dairy cows.

in research involving different animal species such as mini pigs (Brainerd et al., 2010), pigeons and alligators (Gatesy et al., 2010), dogs (You et al., 2001; Tashman and Anderst, 2003; Wachs et al., 2016), rabbits (Henderson et al., 2014), and horses (Panagiotopoulou et al., 2016), but not cattle.

The 3D animations can be accomplished in various ways. Marker-based registration represents the gold standard (Brainerd et al., 2010; Figure 2, top). It requires surgical implantation of at least 3 radio-opaque markers into each bone of interest to facilitate 3D animations. Although marker-based registration offers high accuracy (0.12 ± 0.08 mm; Miranda et al., 2011), noninvasive markerless techniques ought to be applied to live animals for animal welfare reasons and to enable evaluation of regions that are surgically difficult to access. For noninvasive animation, 2 animation techniques are available: a semi-automatic (Figure 2, middle; Miranda et al., 2011) and a manual technique (Figure 2, bottom, Scientific Rotoscoping; Gatesy et al., 2010). Neither of these noninvasive animation techniques have been validated for the bovine distal extremity with special regard to the artiodactyle anatomy.

Because of its high resolution and high accuracy, HFK is suitable for precisely analyzing the changes of skeletal structures during movement (Brainerd et al., 2010). Applied to the bovine distal limb, HFK may allow visualization and measurement of pathological alterations of claw biomechanics. These new insights may improve the prevention of lameness; for example, by investigating claw–floor interactions on various types of flooring. They may also enable the evaluation of influences of claw-trimming methods on claw biomechanics. Digital motion as well as the influence of ground conditions may be measured precisely.

Hence, the first aim of this study was to establish the application of biplane HFK to bovine distal limb and to provide a proof of concept in describing 3D bone movement reliably and precisely. The second aim was to evaluate 2 noninvasive markerless animation techniques (semi-automatic and manual technique) with regard to future applicability in live dairy cows. We hypothesize that (1) the accuracies of the two markerless animation techniques do not differ significantly; (2) the accuracy measurements of left and right phalanges do not differ significantly; and (3) both noninvasive, markerless animation techniques can match all 6 phalanges throughout simulated steps.

## MATERIALS AND METHODS

The phalanges of 5 isolated distal limbs of German Holstein dairy cows were analyzed with 3 different animation techniques with regard to bias and precision (Figure 3).

### *Specimen Preparation*

Three left and 2 right bovine distal forelimbs, separated just below the metacarpal joint, were obtained from a local abattoir. Only limbs that were externally intact and showed no pathologies in computed tomography (**CT**) scans were included in this study. The digits were classified according to their proximity to the image intensifiers and cameras. The phalanges were defined as left (**L**; far from the camera) and right (**R**; closer to the camera). Claw length was measured according to Nuss et al. (2011) (mean left claw length: 116.02 mm; mean right claw length: 119.48 mm). Proximal and middle phalanges were surgically implanted with 3 spherical tantalum markers of 1-mm diameter (X-medics Scandinavia Smba, Frederiksberg, Denmark), and distal phalanges were implanted with 4. Markers were inserted through 2-mm venous catheters (Braun, Melsungen,