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Technical note: Development and evaluation of a standard operating procedure for ultrasound-based measurements of teat canal dimensions in dairy cows

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ABSTRACT

The objectives were to (1) develop a standard operating procedure (SOP) for ultrasound-based measurements of teat canal dimensions and (2) determine the precision of ultrasound-based measurements of teat canal dimensions in dairy cows. Teat scans (n = 64) of the right hind and left front teats from 16 cows were obtained with a portable ultrasound device before and after machine milking. Teat dimensions measured were teat canal length (TCL), teat canal diameter at the proximal end of the teat canal (TCDPROX), teat canal diameter at the midpoint between the proximal and distal ends of the teat canal (TCDMID), teat canal diameter at the distal end of the teat canal (TCDDIS), teat end diameter at the midpoint between the proximal and distal ends of the teat canal (TMD), and teat canal cross-sectional area (TCLAREA). An SOP was developed, reviewed, and modified by 3 operators. Measurements were performed by the same 3 operators using an open source software program. To assess the effect of implemented modifications and the precision of ultrasound-based measurements, concordance correlation coefficients (CCC) were calculated to determine interoperator reproducibility and intraoperator repeatability. Through modifications in the SOP, interoperator CCC increased from 0.45 to 0.86 for TCL, from 0.14 to 0.66 for TCDPROX, from 0.24 to 0.66 for TCDMID, from 0.06 to 0.56 for TCDDIS, from 0.64 to 0.91 for TMD, and from 0.17 to 0.64 for TCLAREA. Intraoperator CCC over all operators were 0.91 for TCL, 0.73 for TCDPROX, 0.80 for TCDMID, 0.69 for TCDDIS, 0.94 for TMD, and 0.75 for TCLAREA. Our results indicate that measurements of teat canal dimensions can be conducted with satisfactory precision when following an SOP. Development and strict implementation of an SOP has the potential to decrease inadvertent variability of ultrasound-based measurements among operators for ultrasonographic assessment of teat dimensions.
Key words: teat canal, repeatability, reproducibility, ultrasound

Technical Note

The teat canal represents the first line of defense against mastitis-causing pathogens entering the mammary gland (O'Shea, 1987). Its anatomical structure and dimensions have therefore been the subject of multiple studies using invasive techniques, such as a modified cannula (Grindal et al., 1991; Lacy-Hulbert and Hillerton, 1995) and contrast radiography (McDonald, 1968a,b, 1975). Ultrasonography (**USG**) has attained increased popularity in bovine medicine within the last decades (Buczinski and O'Connor, 2016). Because of its noninvasive nature, it is perceived as a valuable diagnostic tool for clinical applications in individual cows (Braun, 2016a,b,c) and production medicine (Bollwein et al., 2016; Ollivett and Buczinski, 2016; Teixeira et al., 2017). Due to permanent improvements in ultrasound technology, some authors described the ultrasonographic appearance of the bovine teat canal (Franz et al., 2001). Subsequently, associations between teat canal dimensions as assessed with USG and cow characteristics, risk of mastitis (Klein et al., 2005), and milking technique (conventional vs. automatic milking; Khol et al., 2006) have been investigated. A further application of USG of teat canal dimensions could be examining the influence of machine milking on teat canal diameter as a measure of teat canal penetrability.

The teat canal is a delicate structure only several millimeters long (11–17 mm) and a few millimeters wide (1.0–3.3 mm; Weiss et al., 2004; Klein et al., 2005; Khol et al., 2006). These small dimensions make it difficult to obtain reliable ultrasound-based measurements that represent differences between associated factors rather

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than measurement error. To make valid inferences from studies incorporating ultrasound-based measurements (i.e., teat canal dimensions), measuring techniques require high precision (interoperator reproducibility and intraoperator repeatability). Therefore, the objectives of this study were to (1) develop a standard operating procedure (**SOP**) for ultrasound-based measurements of teats in dairy cows that ensures low variability when measuring relevant teat dimensions and (2) determine its interoperator reproducibility and intraoperator repeatability.

This study was conducted in January and February 2017 at the Teaching Dairy Barn of the College of Veterinary Medicine, Cornell University (Ithaca, NY). All procedures were approved by the Cornell University Institutional Animal Care and Use Committee. Holstein cows (n = 123) were housed in 2 freestall pens with sand bedding and fed a TMR that met or exceeded the National Research Council (2001) requirements. Cows were milked 3 times daily (0400, 1100, and 1900 h) in a 2 × 10 parallel milking parlor (P2100, DeLaval International AB, Tumba, Sweden). To improve ease of ultrasonographic imaging and minimize potential confounding of teat dimensions by peripartal udder edema, inflammation, or injury, cows with the following inclusion criteria were eligible for enrollment: ≥30 DIM, free of clinical mastitis for the last 4 wk, and no udder abnormalities such as nonlactating quarters or teat injuries. A convenience sample of 16 cows was selected from a list of 80 eligible cows. Cows were in their first (n = 2), second (n = 6), third (n = 3), fourth (n = 2), fifth (n = 2), or sixth (n = 1) lactation and between 42 and 392 DIM. The average (mean ± SD) previous-lactation 305-d mature equivalent milk yield was 13,917 ± 2,383 kg and ranged from 9,081 to 17,622 kg.

Teat USG of the left front and right hind teats was performed by 1 trained investigator with a portable ultrasound device (Sonosite Edge, Sonosite Inc., Bothwell, WA) and a 5- to 10-MHz linear array transducer (L52, Sonosite Inc.). Teat scans were taken 90 s after initiation of premilking udder preparation immediately before milking unit attachment and within 90 s after milking unit detachment in the milking parlor. To avoid teat deformation, teats were immersed in warm (30°C) 0.5% chlorhexidine solution (Vet One, Boise, ID) as described by Bruckmaier and Blum (1992). To minimize interference with the dairy's milking routine, ultrasonographic scanning was performed during 3 evening (1900 h) milking sessions (5, 5, and 6 cows, respectively). During teat scanning the entire width of the teat canal was screened from one side to the other by carefully moving the container on a lateromedial line, aiming for the widest diameter. When a scan of the teat was achieved with all structures clearly visible, an image was stored. All teat scans were stored on the integrated flash drive in JPEG format and transferred to a computer with a USB for image processing.

Images were evaluated visually in Windows Photo Viewer (Microsoft Corp., Redmond, WA) and classified by the first author into 1 of 3 categories as follows: good (all boundaries clear and distinct), moderate (1 blurry boundary), or poor (more than 1 blurry boundary). Only images classified as good (n = 47) or moderate (n = 13) were considered for inclusion. Measurements were performed by 3 independent operators using an open source software program (ImageJ, National Institutes of Health, Bethesda, MD). Teat dimensions measured were teat canal length (**TCL**), teat canal diameter at the proximal end of the teat canal (**TCDPROX**), teat canal diameter at the midpoint between the proximal and distal ends of the teat canal (**TCDMID**), teat canal diameter at the distal end of the teat canal (**TCDDIS**), teat end diameter at the midpoint between the proximal and distal ends of the teat canal (**TMD**), and teat canal cross-sectional area (**TCLAREA**). An SOP was developed and evaluated by the 3 operators in 2 separate trials in the following manner. Based on information compiled from the scientific literature referring to measurements of teat (canal) dimensions, a draft SOP was written.

In a 3-h training session, the 3 operators conducted the measurements together using the draft SOP in a total of 10 images that were derived from the same herd using the procedure described herein. When disagreement in the identification of teat dimensions occurred, the 3 operators reviewed the definitions of respective boundaries until agreement was reached, and the draft SOP was modified accordingly.

In trial 1, the modified draft SOP was evaluated by assessment of interoperator reproducibility (agreement between measurements performed by different operators). For this purpose, 25 images were randomly selected with the random number function in Microsoft Excel (2013 version, Microsoft Corp.) from a total of 60 eligible images. Identification number, teat position, and time point of teat scanning were removed. Consequently, the images were assigned a random number. Measurements were performed independently by the 3 operators using the modified draft SOP within 1 wk.

After trial 1, the modified draft SOP was reviewed by the 3 operators, possible sources of inadvertent variability (i.e., difficulties and discrepancies in the identification of boundaries) were discussed, and adjustments were implemented. Criteria that were added after trial 1 and a detailed description of the final SOP are provided in the supplemental materials. Supplemental Figure S1 (https://doi.org/10.3168/jds.2017-13326) illustrates measured teat dimensions.