Ultrasonographic appearance of edema caused by injections in the mammary gland attachments of dairy cows

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Objective—To determine the ultrasonographic appearance and detectability of edema induced by SC injection of mild silver protein suspension in the mammary gland attachments of dairy cows.

Design—Prospective study.

Animals—6 lactating cows.

Procedure—In each cow, the number of quarters that received injections was randomly assigned. A mild silver protein susoension was injected SC into cranial and caudal mammary gland attachment sites. The number of injections and volume injected were determined on the basis of the appearance of the mammary gland and the desired subjective visual effect. Seventeen sites were chosen for injection and 7 sites did not receive injections. Ultrasonographic images were obtained 1 day prior and 6 days after injections were started. Cows received injections 1, 3, and 5 days after initial sonography. The sonographer was unaware of which sites received injections.

Results—Ultrasonography revealed alternating hypoechoic and hyperechoic bands at injection sites. Certain injections caused the intimal surface of the subcutaneous abdominal vein to develop a corrugated appearance. All injection sites were correctly identified ultrasonographically (100% sensitivity, 100% specificity) with a positive and negative predictive value of 1.0.

Conclusions and Clinical Relevance—Results suggest that mild silver protein suspension injected SC to enhance the appearance of the mammary glands of dairy cows can be readily detected by ultrasonography. Detection of injection sites should be made on the basis of the distribution and ultrasonographic appearance of edema. (*J Am Vet Med Assoc* 2002;221:408–410)

RUMINANTS/ POULTRY

Cows used for exhibition are prepared to optimize their external appearance. There is concern that certain forms of artificial augmentation are unethical and affect the quality and fairness of cattle shows. Until recently, infusion of gas into the udder was used to make the udder appear larger.[1,2] Results of previous research indicate that ultrasonographic techniques have high sensitivity and specificity for detection of infused gas in the udder.[3] Since that report was published, there has been a substantial decline in the detected prevalence of this practice at national and international exhibitions; the overall prevalence of cows with detectable gas in the udder at major shows decreased to 0% in 2001. Informal reports indicate that foreign materials are presently injected into sites of cranial and caudal udder attachments of show cows to augment the contour of the udder by causing localized edema. The purpose of the study reported here was to determine the ultrasonographic appearance and detectability of edema caused by injections in the udder attachments.

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Materials and Methods

Six cows from a registered commercial Holstein milking farm were chosen on the basis of milk production, good udder conformation, and tractable behavior. No clinical or visual evidence of mastitis, external wounds or insect bites, or other medical problems were noted prior to the procedures. Milk production and general behavior were monitored twice daily by farm personnel. This research protocol was approved by World Dairy Expo and Holstein USA, and the owner provided consent for the use of the cows.

Subcutaneous injections were made by an individual with experience in preparing cows for national exhibitions. The goal of the injections was to prepare the cows as if for an exhibit on day 6 of the study. As determined on the basis of the injector's experience, this would require a series of injections on days 1, 3, and 5. The goal was to inject sufficient amounts of material to cause flattening of the normal skin folds in these locations without creating a lump.

Each cow was evaluated for conformation. The dose per injection and number of injections per site were determined on the basis of the cow's conformation and the desired subjective visual effect. Possible injection sites in each cow were the right and left cranial and caudal udder attachments. The injection sites in the caudal udder attachments were high on the udder, adjacent to the cleft formed by the junction between thigh musculature and the udder. The cranial injection sites were in the region of the subcutaneous abdominal vein immediately cranial to the most cranial point of each front quarter.

Injections were made with a 1-ml (tuberculin) syringe and a 25-gauge 5/8-in needle. The injection suspension was formed by reconstituting silver protein[a] with 0.9% NaCl solution (15% wt/vol) and filtering through a 0.22-µm syringe filter.[b] Seventeen sites were randomly chosen for injection, and 7 sites did not receive an injection. Injection sites were cleaned and prepared with isopropyl alcohol. At each injection site, the injection needle was directed into 1 to 4 different locations, and 0.25 to 0.5 ml was injected each day in each location. For the 3 injection days (cumulative), total dose per location ranged from 0.25 to 1.5 ml and total dose per site ranged from 1.0 to 3.5 ml.

Ultrasonographic examinations were made with a 7.5-MHz rectilinear transducer and machine system[c] and performed before injection (day 0) and 1 day after completion of injections (day 6). Each cow was evaluated on day 6 for skin trauma, gross swellings, reddening, or localized increased

warmth associated with injections. Images obtained on day 0 established the baseline normal sonographic features and verified that no edema was present prior to injections. All machine settings were kept constant throughout the study. Isopropyl alcohol was used as a coupling agent, and all images were directly digitized. The distribution of edema was mapped for each cow. The sonographer (RTO) was unaware of which sites within each cow received injections. The sonographer subjectively evaluated mammary temperature at each sonographic examination. Milk production during the study was evaluated via examination of appropriate records. General activity of the cows was subjectively evaluated by the owner. A Fisher exact test was performed to determine the positive and negative predictive values for detection of udder edema by the sonographer. A value of $P \leq 0.05$ was considered significant.

Results

By use of ultrasonography, all sites were correctly identified as normal or having received injections, resulting in significant ($P = 0.01$) positive and negative predictive values (1.0). The appearance of the edema caused by the injections was similar to that of physiologic causes of subcutaneous edema. Alternating hypo- and hyperechoic bands were seen, in addition to overall thickening of the subcutaneous layer (**Fig 1** and **2**).

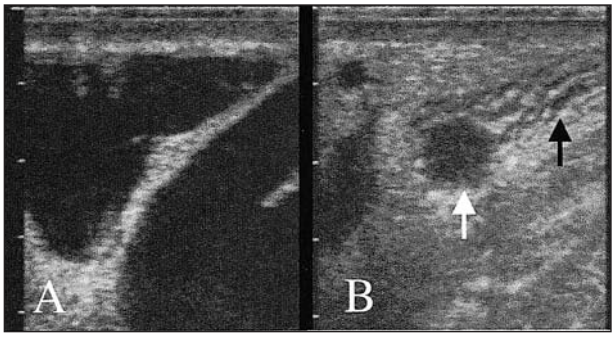

Figure 1—Ultrasound image of a cranial mammary gland attachment site in a cow before injection (A) and 1 day after injections on days 1, 3, and 5 (B) with a silver protein suspension. Notice alternating hyperechoic and hypoechoic bands (black arrow) and corrugated appearance of the intima of the subcutaneous abdominal vein (white arrow).

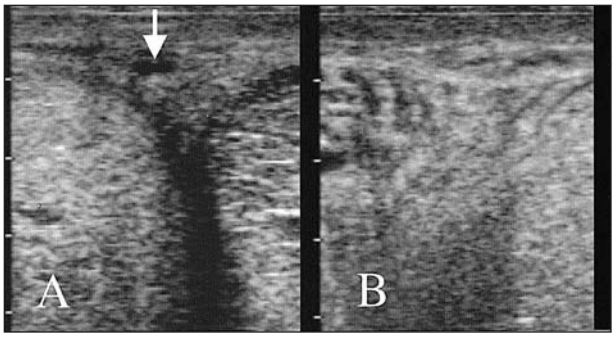

Figure 2—Ultrasound image of a caudal mammary gland attachment site in a cow before injection (A) and 1 day after injections on days 1, 3, and 5 (B) with a silver protein suspension. Notice the superficial perineal vein (A; white arrow) and appearance of thigh musculature (right side of image) and mammary gland tissue (left side of image). The black line originating from the junction of muscular and mammary tissue is normal refraction artifact. In B, notice the alternating hyperechoic and hypoechoic bands, loss of distinct margination of the underlying mammary tissue, and subtle loss of signal strength with increased depth (increased attenuation).

Figure 3—Diagrammatic representations of the extent of edema caused by injections of a silver protein suspension in cranial (left) and caudal (right) mammary gland attachment sites in 6 dairy cows. Heavily shaded areas represent edema in all cows that received injections; lightly shaded areas represent edema in cows that received large injection volumes.

The loose connective tissue adjacent to the banded regions was hyperechoic and hyperattenuating. Increased attenuation of the ultrasound beam was noted deep to the region of edema. Adjacent to the injection sites, edema was limited in distribution (**Fig 3**). Slightly greater distribution was evident in sites that received larger injection volumes, but none of the cows had edema in the most dependent parts of the udder. In 3 cranial injection sites, the contour of the intima of the subcutaneous abdominal vein was irregularly corrugated. No sites were abnormally warm, reddened, or apparently painful at the time of the ultrasonographic examination. No evidence of skin trauma associated with the injection needles or gross swellings were noted in any site. The injections created the desired effect on the cosmetic appearance of the udders. No change in milk production or general activity was noted during the study.

Discussion

Ultrasound is a valuable clinical imaging tool for detection of diseases in the udders of milking cows.[4,5] Typically, there is little gas or mineralization to obscure an ultrasound signal. Detection of masses and other lesions has been documented, and ultrasound can be used in livestock exhibitions for detection of iatrogenic udder abnormalities.

Ethical consideration of cows used for exhibition has gained support nationally and internationally. Part of such consideration is detection of alterations to the udder or milk. A previous report indicated that ultrasound is sensitive and specific for detection of iatrogenically administered isobutane gas in the udders of cows.[3] The gas, a propellant in a widely available mastitis treatment, was not found 72 hours after infusion. Ultrasonographically, the gas had hyperechoic interfaces with characteristic acoustical shadow or reverberation artifacts. Use of ultrasonography resulted in reduced prevalence of iatrogenic gas infusion in the udders of cows in shows.

The study reported here was initiated because of informal reports of continued efforts to artificially augment the appearance of the udders of cows used for exhibition. The SC injection of irritating foreign substances reportedly produced a localized region of edema. When injection was made into a site where a fold or cleft normally existed, a smoother contour resulted. Although the exact substances, volumes, and sites are not common knowledge because of the clan-

destine nature of the activity, we attempted to emulate conditions at cattle shows. Injections were started 5 days in advance of the imaginary show date, and the person who performed the injections was skilled at this procedure. Given the subjective nature of the task, each cow was treated individually rather than in a standardized manner.

The cranial injection sites were in the region of the subcutaneous abdominal vein immediately cranial to the most cranial point of the udder. The goal of these injections was to smooth the fold between body wall and udder, viewed laterally. The normal appearance of these sites included the distended convoluted anechoic appearance of the subcutaneous abdominal vein and variable amounts of fat and loose connective tissue (Fig 1). After injection, a typical pattern of alternating hyperechoic and hypoechoic bands[6] surrounded by overall increased hyperechogenicity and hyperattenuation was seen. In sites where the injections were made in close proximity to the subcutaneous abdominal vein, the contour of the intima of the vein was irregularly corrugated. This may have indicated spasticity as a result of phlebitis, possibly attributable to direct injection into the vessel wall or a reaction to the foreign substance in the perivascular tissues.

The injection sites in the caudal udder attachment were high on the udder, adjacent to the cleft formed by the junction between thigh musculature and udder. The normal ultrasonographic appearance is a variable amount of fat and loose connective tissue in the shape of a triangle when the transducer is aligned horizontally from left to right (Fig 2). A ventral perineal vessel was imaged in transverse plane in this region. The appearance after injection was similar to that of the cranial injection sites. Alternating hyperechoic and hypoechoic bands with hyperechoic loose connective tissue and varying degrees of hyperattenuation were seen. Often the increased attenuation interfered with evaluation of the underlying muscle or glandular tissues. The distribution of edema was exclusively proximal, and no evidence of edema in more dependent locations was noted.

Injection of a mild silver protein suspension induces a consistent localized region of edema. The edema is similar in appearance to physiologic forms, including postpartum edema. This appearance is caused by the accumulation of abnormal fluid (hypoechoic bands) separating the normal fascia layers (hyperechoic bands). Duration of the edema caused by such injection is not known. The duration is of interest in understanding the time course of effects, but of less importance from an ethical standpoint. If edema is seen and determined to have been caused by an injection, the timing of the injection is immaterial.

The effects of ultrasound frequency were not studied. In this study, a 7.5-MHz rectilinear transducer was used. Use of a lower-frequency transducer would decrease resolution of the images and potentially decrease detectability of edema. Most of the lesions were small (approx 1 cm^2) and within 3 cm of the skin's surface. Some low-frequency transducers are unable to decrease the field size to 6 cm or less, which might cause these lesions to be undetected. The rectilinear transducer has an optimal shape with the transducer cable attached to the side. This is especially useful for the cranial udder attachment where scanning is often performed without direct viewing of the entire site.

On the basis of observations at a recent exhibit, we concluded that edema caused by injection of large volumes may persist for 1 week or longer. Smaller sites of edema may lose the characteristic banded appearance as soon as 2 days after initial detection. Hyperattenuation seems to persist longer than the bands do, but this ultrasonographic finding is more subtle and less readily detected.

Differentiation between physiologic and iatrogenic forms of extramammary subcutaneous edema is important. As judged on the basis of clinical observations, the various physiologic forms of edema in the caudal mammary glands do not have a distribution that extends as far dorsally as typical sites of injection. Additionally, the primary site of edema formation in cows after parturition and that in cows that have not been milked for 12 to 30 hours is the median intermammary groove. When severe, the edema extends more laterally. In the cranial mammary glands the primary site of physiologic edema is a horizontal line through the middle of the gland. When severe, the edema is confluent caudally with edema in the rear quarters and extends cranially to the region of the subcutaneous abdominal vein. However, in such instances, perhaps because the inciting cause is not injection of a foreign substance, no evidence of corrugation of the intimal surface of the subcutaneous abdominal vein has been reported or noted clinically. Therefore, except in the most severe instances of physiologic edema, the differentiation between iatrogenic and physiologic edema is based on distribution. Diagnosis of an injection in a cattle exhibition setting is made on the basis of detecting characteristic edema (alternating hyperechoic and hypoechoic bands) in a typical site and lack of confluence with physiologic edema.

[a]Silver Protein (Silver Nucleate), Mild Spectrum, Gardena, Calif.
[b]Millex Sterile Syringe Filter Unit, Millipore, Bedford, Mass.
[c]SSD 500V, Aloka, Wallingford, Conn.

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