J. Dairy Sci. 101:1–8
https://doi.org/10.3168/jds.2017-13346

Technical note: Evaluation of fine needle aspiration cytology for the diagnosis of fatty liver in dairy cattle

P. Melendez,*[1] M. Whitney,* F. Williams,* P. Pinedo,† D. Manriquez,† S. G. Moore,‡ M. C. Lucy,‡ P. Pithua,* and S. E. Poock*
*College of Veterinary Medicine, University of Missouri, Columbia 65211
†Department of Animal Sciences, Colorado State University, Fort Collins 80521
‡Division of Animal Sciences, University of Missouri, Columbia 65211

ABSTRACT

Fatty liver is a common condition affecting dairy cattle during the periparturient period, characterized by a pathological accumulation of triglycerides (TG) in the hepatocytes. The objective of this study was to evaluate the diagnostic potential of fine needle aspiration cytology in fresh liver specimens using liver TG concentrations as a gold standard. Fifty-seven liver samples from Holstein cows were collected during processing at a slaughterhouse. Tissue and fine needle aspirate samples were obtained from the parietal upper portion of the caudate lobe. Two samples of liver tissue were collected with a 16 gauge × 15 cm biopsy needle for histological and TG concentration assessment. A third sample was collected for cytology using an 18 gauge × 5.08 cm needle. The contents of the needle were transferred to a glass slide, spread, and air-dried. Liver samples were assayed by colorimetry/fluorimetry to determine TG concentrations. Concentrations of TG <2% were considered normal. Histological and cytological evaluations were conducted by 2 different pathologists blind to the visual classification. Sensitivity (Se) and specificity (Sp) were calculated. Cytology had a Se and Sp of 73 and 85%, respectively. Histopathology had a Se and Sp of 45.9 and 100%, respectively. The likelihood of having higher scores for histopathology and cytology increased as a function of liver TG content (mg/g).
Key words: fatty liver, cytology, histology, triglycerides

Technical Note

Hepatic lipidosis, also known as fatty liver, is a common condition affecting dairy cattle during the periparturient period, characterized by a pathological accumulation of triglycerides (**TG**) in the hepatocytes (Herdt, 1988). This condition develops secondary to the typical negative energy balance experienced by the dairy cow during the early postpartum period, especially in those individuals with a high body condition at parturition. This hepatic infiltration is a consequence of fat mobilization from adipose tissue, which leads to an increase in fatty acids (**FA**) that are transported to the liver (Herdt, 2000; Bobe et al., 2004; Gerspach et al., 2017). When FA release is severe due to adverse conditions, such as obesity, disease, and poor transition feed management, excess FA in the liver are re-esterified to TG. The rate of liver export of TG as very low density lipoprotein is a slow mechanism in cattle; therefore, the net outcome is a pathological TG accumulation leading to fatty liver syndrome (Herdt, 1988; Bobe et al., 2004).

At present, there is no biochemical diagnostic test that is accurate, fast, and reliable for the diagnosis of this condition (Herdt, 1988; Kalaitzakis et al., 2010). Hepatic TG content can be determined by fat extraction, histopathology, or flotation of liver biopsies obtained in live animals (Herdt, 1988; Bobe et al., 2004). However, liver biopsies are invasive and associated with risks of hemorrhage, infection, and adhesions (Bobe et al., 2004; Swecker, 2014). Another technique for the diagnosis of hepatic lipidosis in cattle is transcutaneous ultrasound; nevertheless, this methodology has low sensitivity (**Se**) when TG contents are <10%. This method is therefore more accurate for the diagnosis of severe fatty liver (TG >10%; Haudum et al., 2011; Rafia et al., 2011; Starke et al., 2011; Weijers et al., 2012).

The use of fine needle aspiration cytology (**FNAC**) for the diagnosis of fatty liver in cattle and other species has been described (Hoff and Cote, 1996; Weiss and Moritz, 2002); however, it has not been extensively evaluated in dairy cows. Fine needle aspiration cytology is technically feasible in clinical practice, being minimally invasive and inexpensive, and having a rapid turnaround time compared with biopsy (Komemushi et al., 2015). Consequently, the aim of this study was to evaluate the diagnostic potential of FNAC for hepatic lipidosis using liver TG extraction as a gold standard.

Received June 17, 2017.
Accepted January 14, 2018.
[1]Corresponding author: melendezp@missouri.edu

Sampling was conducted from a slaughterhouse located in Green Bay, Wisconsin. Livers were selected according to the following criteria: (1) originated from adult mature cows, and (2) no observable evident lesions, such as abscesses, tumors, cysts, and so on. To obtain variability regarding the TG content of the livers, a visual scoring based on surface color was performed to classify the organs. Considering a 2-sample proportions test, with a difference in probabilities of 50% and 95% of confidence and 80% of power, a total sample size of 38 livers (19 visually abnormal and 19 visually normal) was determined. To obtain enough variability, 3 classes were considered and 19 livers per classes were sampled. Class 1 was defined as a normal liver with a homogeneous maroon color. Class 2 was defined as a liver with moderate yellow-pale color, and class 3 was defined as a liver with an extreme yellow color.

Eligible livers (n = 57) were removed from the processing line and placed on a metal table to proceed with the sampling procedure. Both tissue and needle aspirate samples were obtained from the parietal upper portion of the caudate lobe. This area was sampled because it is the normal section for conducting liver biopsies in live adult cattle (superior part of the 12th right side intercostal space). Two pieces of liver were collected with a biopsy tool (16 gauge × 15 cm; Tru-Cut biopsy needle, Jorgensen Laboratories, Loveland, CO). One piece was stored in a plastic tube with 10% formalin for histologic evaluation. The second piece was stored in a tube without preservation medium and placed immediately in a plastic cooler with dry ice for TG extraction. A third sample for cytology was obtained using an 18 gauge × 5.03 cm needle. Half of the needle was introduced immediately next to the area where the liver biopsies were obtained followed by aspiration with a 20-mL syringe. The content collected inside of the needle was spread on a glass slide and air-dried.

Biopsy samples stored in formalin and liver smears were submitted for histologic and cytologic evaluation, respectively, at the College of Veterinary Medicine, University of Missouri. Liver samples for TG extraction were frozen at −80°C until the analyses were performed.

Triglyceride extraction was used as the gold standard for comparison with both microscopic techniques. Approximately 100 mg of liver tissue was obtained from biopsies. The amount of TG per gram of wet tissue was measured using a TG quantification kit (ab65336; Abcam, Cambridge, MA) that uses a colorimetric methodology. Tissue sample processing was according to the manufacturer's instructions (http://www.abcam.com/triglyceride-assay-kit-quantification-ab65336.html). The liver samples were weighed and placed in 1 mL of in 5% NP40 in water and homogenized by using a Tissue Tearor homogenizer (Biospec Products Inc., Bartlesville, OK). The samples were then heated to 80 to 100°C in a water bath for approximately 5 min, cooled to room temperature, and then heated again. The homogenates were then centrifuged at 20,000 × g for 2 min at 23°C and the supernatant was diluted 10-fold in water. Ten microliters of the diluted supernatant was assayed. In this assay, TG are converted to free FA and glycerol; the glycerol is then oxidized to generate a product which reacts with the probe to generate color (λ = 570 nm) and fluorescence (excitation/emission = 535/587 nm). The assay can detect 2 pmol to 10 nmol (2 μM to 10 mM range) of TG. Sample and background assays were performed and background was subtracted from the sample values. The assay gave results in nanomoles per well, which was corrected for sample dilution. The nanomoles of TG were converted to mass of TG by using the equation 1 nmol = 850 ng of TG. The weight of TG was then expressed as a percentage of the wet tissue weight of the starting sample.

Normal livers were defined as having a TG content of <2%. Fatty liver was defined as TG content of ≥2% (Petit et al., 2007; Gross et al., 2013; Crookenden et al., 2016). This assumption was considered because subclinical molecular and pathological changes of the liver start as early as with a content of TG ≥2% (Gross et al., 2013; Crookenden et al., 2016). Biopsy samples for histopathology were fixed in paraffin wax and stained with hematoxylin and eosin. The evaluator was a trained anatomic pathologist from the College of Veterinary Medicine, University of Missouri, board certified by the American College of Veterinary Pathologists. The evaluator was blind to the visual classification score of samples. Microscopic assessment was based on a score of 0 to 4 related to cytoplasm fat infiltration (Figure 1). Histologically, hepatocellular lipid appears as a clear round vacuole. The vacuoles are clear because lipid is removed in routine processing of tissue for histopathology, such as the stain with hematoxylin and eosin. In addition, glycogen accumulation in the liver can lead to variable degrees of pallor and swelling. Microscopically, hepatocytes are swollen with lacy vacuolated cytoplasm. Unlike lipid vacuoles, glycogen vacuoles are poorly defined and hepatocyte nuclei are not displaced to the periphery of the cell (Brown et al., 2017).

Slides with needle aspirate smears were processed in the clinical pathology laboratory of the Veterinary Medical Diagnostic Laboratory. Slides were stained with a modified Wright-Giemsa stain using an automated slide stainer (Hema-tek model 4488CE, Siemens Healthcare Diagnostics Inc., Tarrytown, NY) and examined and classified by a clinical pathologist board certified by the American College of Veterinary Pathologists. The clinical pathologist was blind to the visual classification score of the samples and histopathologic