Liver biopsy procedure and indications

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Abstract

Liver biopsy sampling and subsequent analysis for mineral content can be a valuable diagnostic tool for micronutrient associated illness in cattle. Although there may be some apprehension when using the blind technique, the procedure has little risk of adverse consequences. Utilizing proper biopsy site preparation and appropriate landmarks reduces risk of complications while optimizing the probability of obtaining a sample large enough for micronutrient evaluation. Liver samples are appropriate for evaluating toxicity associated with cobalt, copper, manganese, selenium and zinc and deficiency associated with copper, manganese, selenium and zinc.

Key words: liver, biopsy, cattle, copper, selenium

Liver biopsy background

Biopsy instruments

Liver biopsy sampling and subsequent analysis for mineral content can be a valuable diagnostic tool for micronutrient-associated illness in cattle. Historically, liver biopsies to evaluate vitamin A levels were obtained with a large instrument that required an incision through the skin and body wall or a laparotomy.[1,2] This resulted in a rather large core sample weighing approximately 2 to 8 grams. Current techniques utilize a much smaller instrument like a TruCut soft tissue biopsy needle[3] or a True Cut style 16-gauge soft tissue biopsy needle with a 1 mm x 20 mm specimen chamber.[4] Another instrument, the Schackelford-Courtney bovine liver biopsy instrument is capable of acquiring a 150 mg sample with one biopsy.[3]

Author's experience with adverse outcomes

Although there may be some apprehension when using the blind technique, the procedure has little risk of adverse consequences. The author has completed the blind sampling technique to acquire liver samples for mineral analysis on several hundreds of cattle ranging from 12- to 26-week-old dairy calves and 6- to 9-month-old beef steers to adult beef cows with an adverse experience/severe complication reported in only one 8-month-old beef steer a couple of months after the sampling procedure. Although, pain evaluation was never included as an outcome in any of the investigations, there was no evidence of overt pain associated with any of the biopsies. Several of the calves had multiple liver biopsies acquired over the course of several weeks depending upon the nature of the study or investigation.[4]

Evaluation of adverse outcomes in beef cattle

In 2001, Rogers et al. published the results of 2 trials evaluating the consequences of liver biopsies using the blind technique and a Schackelford-Courtney bovine liver biopsy instrument. In trial 1, 60 steers weighing an average of 871.2 lb were evaluated over an approximately 120-day feeding period with 31 undergoing the biopsy procedure. In trial 2, 66 cross bred heifers weighing an average of 563 lb were evaluated over a 112-day feeding period with 33 undergoing the biopsy procedure. In trial 2 there were no statistically significant outcomes in the measured observations. In trial 1, there were statistically significant differences favoring the steers that were not biopsied with regard to the day 7 average daily gain, dry matter intake and feed-to-gain efficiency. Additionally, there were statistically significant differences favoring the non-biopsied animals in ribeye area, hot carcass weight and dressing percentage. The differences in growth performance were considered minimal by the authors and they concluded that the biopsy procedure was relatively safe and effective.[3]

Evaluation of adverse outcomes in dairy cattle

Two publications evaluating the discomfort of the procedure in adult dairy cows found differing results. In 2012, Molgaard et al., studied 18 Danish Holstein dry cows for evidence of pain through behavioral changes associated with percutaneous liver biopsy. There were numerous untoward behavioral changes that were elevated in association with the liver biopsy. These included outcomes like duration of restless behavior and duration of head shaking. As a consequence, the authors concluded that the anesthetic procedure should be updated and that an analgesic should be included. It is important to note that the biopsy needle used was 14-gauge, but more importantly, the needle was introduced into the liver 13 to 32 times for an average of 17.9 times per animal.[5] In a more recent publication in 2016, investigators in New Zealand evaluated pain associated with percutaneous liver biopsy in 24 non-lactating dairy breed cows. The purpose of the study was to determine whether administration of NSAIDS, ketoprofen and meloxicam specifically, would reduce the pain associated with the biopsy procedures. They were unable to find evidence of pain associated with the procedure in the sham dosed or negative control groups of animals. The investigators concluded that in this study if pain was elicited it was too mild to detect and therefore analgesia in the form of NSAIDs had no beneficial effect. In contrast to the previous study the biopsy instrument utilized in this study was 5 mm liver biopsy trocar that was only introduced once per animal.[6] It is reasonable to conclude that depending on technique, instrument used and number of punctures that analgesia may be warranted.

Liver biopsy procedure

Supplies

Utilizing proper biopsy site preparation and appropriate landmarks reduces risk of complications while optimizing the probability of obtaining a sample large enough for micronutrient evaluation. As with any procedure in a restrained animal, in order to reduce the possibility of injury and to reduce animal discomfort, it is important to be as efficient as possible. Therefore, making sure that one is prepared with the appropriate supplies is essential. The author includes the following items in their liver biopsy kit: clippers equipped with a #40 blade, scrub solution (betadine or chlorhexidine), alcohol, clean 4x4 gauze pads, lidocaine, 6 mL syringe, 18-gauge x 1.5-inch needle, scalpel blade, 14- or 16-gauge True Cut Style soft tissue biopsy needle, sterile gloves, 5 mL plastic screw cap transport tube, and betadine or chlorhexidine ointment. These are personal preferences and many of the items can be replaced without having detrimental outcomes. It is imperative that if the goal is to assess

micronutrients that the tube used for the capture and transport of the liver samples not be contaminated with double-positive charged elements like zinc as that can produce spurious results. Therefore, if for convenience, one was to use a serum tube for collection and transport to the lab, it is imperative that one use a royal blue top tube. Other tubes rubber stopper may contain very low levels of double positive charged elements that will produce spurious results.

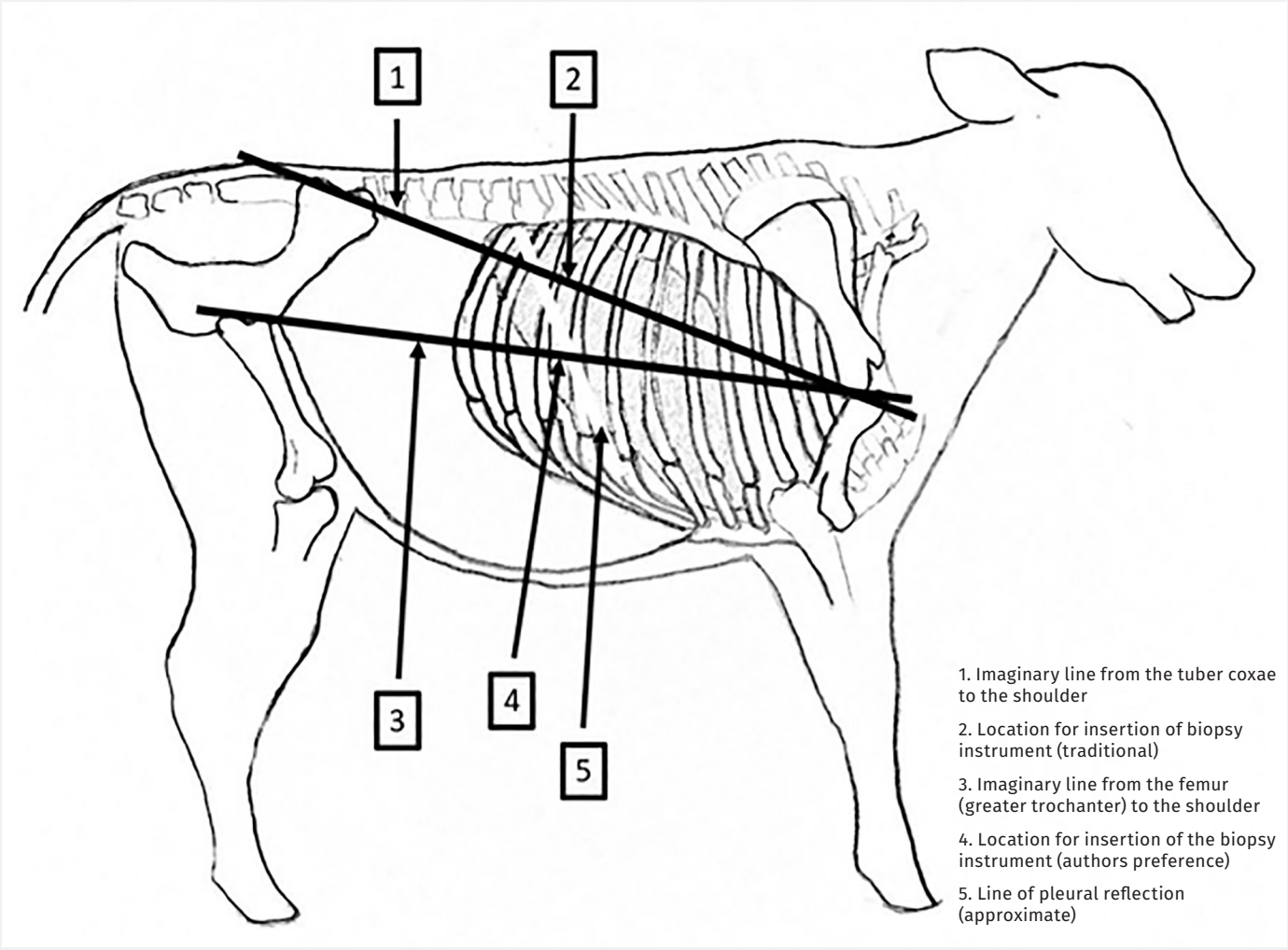
Site location

The author follows a procedure similar to the one detailed in Rogers et al.[3] The first most important first step is to have adequate restraint. For most subjects, this requires a squeeze chute. For smaller calves this may mean chemical restraint through heavy sedation. Next, one must identify the location for percutaneous entry into the abdomen (Figure 1). This may be obvious, but remember to work from the right side, where the liver is located in cattle. Using anatomic landmarks will help to ensure a successful biopsy procedure. First, identify the 10th intercostal space. This is most easily performed by counting backward from the last (13th) rib to find the space between the 10th and 11th rib. Most descriptions direct one to draw an imaginary line between the top of the tuber coxae and the right shoulder. At the point where that line intersects the middle of the 10th intercostal space is the place for percutaneous entry. The author prefers to make an entry point a few centimeters below that point. Instead, imagine a line from the top of the femur (greater trochanter) to the shoulder. The point where the imaginary line bisects the 10th intercostal space would be the target for percutaneous entry (Figure 1). The reason for this adaptation is two-fold. First, there may be less chance of accidentally piercing the lung as needle passes through to the liver. Second, the angle of the biopsy needle is much less severe making manual manipulation of the instrument easier.

Site preparation

The next step is to clip the area for entry. In general, the clipped area is a rectangle centered around the point identified for entry, with the width encompassing at least the back of the 10th rib and the front of the 11th and the length along the intercostal space extending 5 to 6 cm dorsal and 5 to 6 cm ventral to the insertion point identified. The clipped area is then rinsed and wiped with alcohol to remove organic debris and oils. An initial site preparation with alternating scrub and alcohol rinse proceeds. At this juncture is when the lidocaine is administered. One to 2 mL bleb under the skin followed by a 2 to 3 mL instillation in the muscles. This is followed with at least one more preparation with alternating scrub and alcohol. This final site preparation is mostly to allow time for the lidocaine to have an effect. Once the site is prepared, the author lays out the

Figure 1: Landmarks for percutaneous liver biopsy.



biopsy instrument, the scalpel and dons sterile gloves. A small stab incision through the skin is created with the scalpel.

Biopsy acquisition

The biopsy needle is introduced through the incision and underlying muscles. One may feel a slight pop when pushing through the facia into the thoracic/abdominal cavity. The needle should be introduced in a manner such that the target is the opposite (left) shoulder. In practice the angle forward and down does not need to be severe. It can be in many instances almost straight forward. The needle should be introduced until mild constant resistance is felt. The depth is usually 8 cm or less. The device is than manipulated to obtain a sample. Prior to attempting a biopsy, one should familiarize themself with how the instrument functions. It can take some practice to develop the dexterity to use the instrument appropriately to obtain full length samples. Briefly, the entire needle should be advanced together in a smooth fashion once the typical firm resistance is felt the inner trocar/needle structure is advanced while maintaining the outer sleeve in place. Once the inner needle has been fully advanced, it is now held in place while the outer sleeve is advanced in a firm, quick manner. The entire biopsy needle is removed, and the outer sleeve retracted to reveal a liver biopsy sample in the specimen chamber. Using an 18-gauge x 1.5-inch needle, the sample is gently teased from the specimen chamber into the transportation tube. The sampling procedure is repeated a total of 3 to 4 times to obtain a large enough sample for analysis. The final step is to cover the stab incision with a small volume of betadine or chlorhexidine ointment and release the animal from the restraint device.

Post-procedure/peri-procedure considerations

In an abundance of caution, the author recommends keeping sampled animals in an enclosure where they can easily be visualized for 2 hours. Antimicrobials for infection prevention are not recommended unless some unforeseen contamination occurs during the procedure. The author does not commonly use NSAIDs or other analgesics. The available evidence on pain associated with liver biopsies and the effectiveness of NSAIDs are mixed.[5,6] One may hypothesize that administering NSAIDs could lead to increased potential for bleeding associated with the biopsy procedure. The author was unable to find any studies investigating this potential in cattle. There are numerous studies available in the human literature evaluating NSAID administration and soft tissue biopsies. The results of these studies indicate very low relationship with NSAID administration, soft tissue biopsy and bleeding.[7,8]

Indications for liver biopsy in cattle

In addition to research applications, there are numerous clinical diagnostic indications for performing liver biopsies in cattle. Liver samples are appropriate for evaluating toxicity associated with cobalt, copper, manganese, selenium and zinc and deficiency associated with copper, manganese, selenium and zinc.[9]

Cobalt and manganese

Liver biopsies have limited utility in cobalt deficiency unless coupled with vitamin B12, however it can be a good diagnostic in the rare instance of cobalt toxicity.[9,10] Clinical signs associated with cobalt toxicity include excessive urination, defecation, salivation and shortness of breath. These signs are often accompanied by polycythemia, resulting in increased hemoglobin, red blood cell count and packed cell volume.[10] Both deficiency and toxicity with manganese is uncommon. Manganese deficiency in cattle can result in a number of musculoskeletal abnormalities reduced reproductive performance. Toxicity has been associated with decreased feed intake and reproductive performance.[9,10]

Selenium

Selenium deficiency can be diagnosed by measuring blood, serum, liver or kidney selenium concentrations; however, liver selenium concentrations are considered the best indicators of selenium status.[9] The primary role of selenium relates to its role as an essential component of glutathione peroxidase, the enzyme that protects cell and organelle membranes from oxidative damage. Selenium deficiency has been linked to a variety of clinical disease manifestations in cattle. These include nutritional myodegeneration (NMD), decreased reproductive performance, retained fetal membranes, increased disease susceptibility, and ill thrift.[11] Selenium toxicosis is characterized by lameness associated with cracked and deformed hoofs, loss of hair (alkali disease) as well as neurologic manifestations including circling, head pressing, ataxia and convulsions (blind staggers).[9,10] Acute selenium toxicosis can result in death.[10]

Zinc

Tissue levels of zinc are not reliable predictors of zinc status in cattle because there is no real storage pool in the cattle body tissues, therefore serum or blood levels may be a better indicator of zinc status.[9] However, liver does represent a tissue with a reasonable concentration of zinc[9] and may be helpful, if a liver biopsy is already part of the diagnostic workup. Clinical signs and abnormalities present in cattle with zinc deficiency include decreased growth rate, diarrhea, poor appetite, salivation, abnormal hooves, swollen joints and coronary bands, stiff gait, hair loss, parakeratosis, thymic atrophy, lymphoid depletion, decreased disease resistance with specific depression of cell-mediated responses, and decreased reproductive performance.[12] Zinc toxicity is uncommon in cattle.[9] Adult dairy cows can have decreased milk production and feed intake.[10] In young calves, which are more susceptible, one can see diarrhea and polyuria, followed by pica. Arrythmias, convulsions and lack of sensorium may be seen.[9] More commonly in instances of excessive zinc, clinical signs of copper deficiency will manifest.[9,10]

Copper

The liver is the primary copper storage organ. Copper is released from liver stores to maintain blood copper concentrations and essential physiologic functions. Consequently, serum copper concentration may overestimate total body copper stores during copper deficiency and underestimate body copper stores during copper toxicosis. Copper deficiency has been linked to a variety of clinical signs, including pale coat, poor fleece quality, anemia, spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelinization of the spinal cord, impaired reproductive performance, decreased resistance to infectious disease, diarrhea, and generalized ill-thrift.[4] Copper toxicity is very uncommon in beef cattle; however, the author has diagnosed a herd outbreak that resulted in hemolytic crisis and death. This was a result of feeding a diet that largely consisted of poultry litter. Cattle presented for necropsy had abnormal liver and kidneys. Liver and serum samples of herd mates had elevated copper concentrations. Other manifestations associated with copper toxicity in cattle include, depression, anorexia, decreased

milk production, head pressing, ataxia and circling behavior.[9] Over the last decade or more, a syndrome of liver copper accumulation leading to clinical signs associated with copper toxicity progressing to death in some cases has been recognized in dairy cattle across the globe.[13] Population characterization through abattoir samples have been used to characterize the syndrome in the U.K.[14] In that survey, 510 liver samples, 419 from cull dairy cows, were collected and analyzed for copper levels. Over 50% of samples had copper levels above normal and 38.3% of Holstein-Friesian dairy cows and 40% of other dairy cows were above the upper limit of the reference range, which is 30% more than the upper normal limit.[14] In addition to the U.K., this trend of liver copper accumulation in dairy cattle has been recognized in the U.S., New Zealand, the Netherlands and Spain.[13]

Mineral analysis

Atomic absorption spectrophotometry

Historically, metal concentrations in tissues and other matrixes were determined using atomic absorption spectrophotometry. This method is cumbersome and requires substantial samples if there is more than one metal of interest. Each analysis requires a separate lamp and sample preparation. Lamps that emit light at a specific wavelength are required for each metal of interest. For instance, copper determinations require a lamp with a wavelength of 324.7 nm.[4] Different samples preparations are required because the sample is consumed during the atomic absorption analysis.

ICP/MS – inductively coupled plasma-mass spectrometry

Many labs use a modern technique, inductively coupled plasma-mass spectrometry, to determine the concentration of numerous metals with one sample. The Michigan State University Veterinary Diagnostic Laboratory can provide results for cobalt, copper, iron, manganese, molybdenum, selenium and zinc from a single fresh sample.

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