A Surgical Method of Obtaining Liver Biopsies from Adult Swine for Use in Mineral Analysis

J.G. Powell[1], K.E. Washburn[2], C.V. Maxwell[1], E.B. Kegley[1]

Story in Brief

The objective of this study was to determine if liver biopsies of sufficient quantity for trace mineral analysis could be safely obtained percutaneously from adult swine without the aid of ultrasound guidance. Since no previous liver biopsy protocol existed for adult swine, a collaborative effort between researchers from the University of Arkansas and Oklahoma State University investigated the development of an effective liver biopsy technique. Several biopsy instruments were reviewed before settling on the Courtney bovine liver biopsy instrument. This instrument was large enough to get the desired sample size of 400 mg, and had been shown to work in bovine liver biopsies. Twelve healthy pigs with an average BW of 460 lb were used for the study. During the biopsy, the experimental group of pigs were anesthetized and placed in left lateral recumbency. A percutaneous liver biopsy was performed from the right side on each pig based on expected anatomical location of the liver. All experimental pigs underwent a postmortem examination 48 h after the procedure. They were evaluated for any significant lesions that may have occurred during the procedure. The procedure demonstrated successful biopsy results, and the desired amount of liver tissue was retrieved using this technique. No undesirable lesions were found upon examination of the carcass at the conclusion of the trial.

Introduction

The balance and availability of trace minerals in swine has documented effects on various parameters of reproductive efficiency and performance (Mahan et al., 1974, and Wilkinson et al., 1977). Body stores, or reservoirs, of these elements are available in times of demand that may be mobilized for use. The liver serves as one such reservoir for many minerals including copper and zinc, which play vital roles in ensuring optimal reproductive performance (Mahan, 1990). It is documented that reservoir levels of trace minerals fluctuate as sows go through various phases of the production cycle such as gestation, farrowing and breeding (Mahan, 1990). Inefficiencies in reproductive performance can occur if reservoir levels become depleted, and are not adequately restored by dietary or supplemental means (Mahan, 1990). The ability to assess the available level of minerals in reservoirs, such as the liver, in representative pigs from all phases of a swine production unit, facilitates correction of deficiencies or imbalances and allows evaluation of the efficacy of various mineral sources in the breeding herd. Obtaining liver tissue for antemortem mineral analysis has been reported by means of laparotomy (Jensen, et al., 1990) and percutaneous (Simmons, 1971, Van Lith, et al., 1988, Swindle, 1983) biopsy. Performing a laparotomy in a field setting would be problematic due to the necessity of a sterile environment and inhalant anesthesia. In addition, a laparotomy on an adult pig would be labor intensive and time consuming. Detailed reports could not be found describing a successful percutaneous approach on pigs greater than 155 lb with or without the use of ultrasound guidance. However, ultrasound imaging equipment is cumbersome and expensive. In the field setting of a production unit, it would be of benefit to be able to consistently and safely obtain liver tissue without the use of ultrasound guidance. The objective of this study was to determine if liver biopsy samples of sufficient quantity for trace mineral analysis could be safely obtained percutaneously from adult swine without the aid of ultrasound guidance using a large diameter instrument.

[1] Department of Animal Science, Fayetteville

[2] College of Veterinary Medicine, Oklahoma State University, Stillwater

Experimental Procedures

Twelve healthy female pigs were randomly divided into two groups of six. The experimental group consisted of one gilt, two first parity sows, one second parity sow, one third parity sow and one fourth parity sow. The average BW of pigs in the experimental group was 474 lb (range 366 lb to 571 lb). The control group consisted of one gilt, three first parity sows and two third parity sows. The average BW of pigs in the experimental group was 446.5 lb (range 403 lb to 529 lb). All pigs were housed individually and cared for in compliance with Animal Care Protocol # 04004 for swine experimentation issued by the University of Arkansas Animal Care Committee.

All pigs were weighed, and then anesthetized by intramuscular injection of a combination of xylazine (250 mg), ketamine (250 mg) and Telazol (tiletamine 50mg and zolazepam 50mg, Fort Dodge Animal Health, Fort Dodge, Iowa) at the rate of 1 cc/cwt. A local anesthetic of 2% lidocaine hydrochloride (1 cc) was infiltrated in the skin at the site of liver biopsy.

Following the administration of anesthesia, pigs were then placed in left lateral recumbency. The location for liver biopsy was approximately 4.5 inches ventral to the rib cage on the right side, at the level of the 10th rib. The site for liver biopsy was clipped, infiltrated with lidocaine and surgically prepared with 2% chlorhexidine scrub. A stab incision was made through the skin at the site of lidocaine infiltration with a #15 scalpel blade. In the control group, this stab incision was subsequently closed with one cruciate suture of 1-0 Braunamid (Jorgensen Laboratories, Inc., Loveland, Colo.). In the experimental group, liver biopsy was performed and then the incision was closed in the same manner. The liver samples were obtained using a Courtney bovine liver biopsy instrument (Sontec Instruments, Inc., Englewood, Colo.). The instrument was passed through the skin incision to a depth of 4 in with the biopsy chamber in the fully retracted position. Proper depth of insertion was based on previous pilot trials using ultrasound to measure from skin surface to liver in pigs of similar size. At this point, the biopsy chamber was extended into the liver tissue. While holding the chamber stationary, the outside portion was briskly moved over the chamber to cut out a core of liver tissue. The entire instrument was removed, and the chamber was extended to reveal the removed tissue. The liver tissue was placed on an electronic scale (Denver Instrument, Arvada, CO) to determine weight.

Following the biopsy procedure, all pigs were given a penicillin injection at a rate of 2 cc/cwt. During the 2-day study, all pigs were monitored for general health. Forty-eight hours after the procedure, the six pigs of the experimental group were euthanized by captive bolt pistol and exsanguination. The six experimental pigs were necropsied and examined for significant lesions and location of biopsy in the liver. On post-mortem examination, the abdominal cavity was examined for evidence of hemorrhage, gross abdominal cavity contamination and accidental puncture of other organs. The entire liver was removed, and the exact site of liver puncture was recorded.

For mineral analysis, liver mineral concentrations were determined after drying and wet ashing the biopsy samples. Samples were thawed, transferred to 50-mL polypropylene tubes and dried for 48 hours in a 100°C gravity convection oven. Dry sample weights were obtained. Samples were digested in 15 mL of trace metal grade nitric acid at 115°C for 1 hour in the polypropylene tubes, then brought to a constant volume of 45 mL with deionized water and analyzed by atomic emission spectroscopy (Spectro ICP, Model FSMEA85D, Fitchburg, MA). The laboratory required approximately 400 mg of tissue for this analysis.

Results and Discussion

Liver biopsy samples were successfully obtained from all six experimental pigs. An average of 440 mg (range 418 – 486 mg) of liver was obtained for mineral analysis. Due to the friable nature of the porcine liver, an average of two samples using the Courtney bovine liver biopsy instrument were required to obtain enough tissue for mineral analysis. Necropsy results indicated no significant complications occurred as a result of the biopsy technique. No adverse reactions to the anesthetic agents were noted during or following the procedure. Results of trace mineral analysis for blood and liver are shown in Table 1. All 12 pigs were noted to be in good health for the 48-hour study period.

On gross post-mortem examination, there was no evidence of gross abdominal hemorrhage or inadvertent puncture of undesirable organs. The puncture sites in four of the six pigs were observed in the ventral one-third of the right medial liver lobe. However, in the 4th parity sow, the liver biopsy sites were observed in the left medial lobe of the liver. The average distance from the skin surface to the liver surface was 2 3/4 in.

Results of our study suggest that a large bore instrument capable of obtaining sufficient quantities of liver tissue for mineral analysis can be used percutaneously, without ultrasound guidance, in large, adult swine. It was not the intent of this study to evaluate the mineral levels in these pigs, but rather the feasibility of obtaining enough tissue to do so. Therefore, only the quantity of the various minerals is reported, without interpretation to demonstrated efficacy of the procedure.

The pigs in this experiment were not fasted prior to anesthesia. In a field setting within a large production unit, fasting animals for the appropriate time prior to anesthesia may be impractical; therefore, the authors thought the risk of aspiration during a relatively short procedure was minimal. In addition, in previous trials performed at the University of Arkansas, in similar size pigs that were fasted 24 hours prior to anesthesia, the gall bladder was greatly enlarged via ultrasound observation. The risk of accidental puncture of a distended gall bladder was thought to be greater than the risk of anesthetic complications.

Although it is certainly possible that this technique may not be useful for all sizes and genders of swine, according to anatomical texts, this "window" of percutaneously accessible liver should be present (Dyce, et al., 1987). However, it is recommended that trials using ultrasound recognition and exact location of the liver be conducted prior to percutaneous biopsy in pigs smaller than the ones recorded within this study.

Overall, although the numbers in this study were small, it appears that this procedure can be performed in a field setting with minimal risk to the pigs. However, this procedure does require practice, aseptic technique, and post sampling antibiotics; therefore, it is not without some degree of risk to the pig. Avoiding laparotomy or post-mortem sampling of pigs of this size to obtain trace mineral status would be beneficial in order to correct imbalances or deficiencies or to determine efficacy of a specific mineral source.

Implications

Obtaining percutaneous liver biopsies of sufficient quantity for mineral/element analysis can be performed safely without the aid of ultrasound using the technique developed. This procedure will circumvent the need to obtain liver tissue via cumbersome methods such as necropsy or laparotomy in large, adult pigs. The use of this technique will allow researchers to more accurately evaluate the trace mineral reservoir status of swine in all phases of the production unit.

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Table 1. Mineral levels in swine liver tissue obtained from percutaneous biopsy with a large bore instrument. Normal range would be values from liver that could be acquired via laparatomy or postmortem sampling.

Pig no.	Copper (ppm)	Zinc (ppm)	Iron (ppm)	Phosphorus (ppm)
223	94	83	1,937	4,740
325	84	25	1,678	2,134
390	102	215	1,044	8,585
535	36	317	1,518	10,220
410	164	155	1,096	8,037
349	27	112	866	5,989
Normal Range	5-100	40-90	100-200	12,000-14,000