

LAPAROSCOPIC BIOPSY TECHNIQUE OF LIVER AND SPLEEN IN BUFFALO CALVES

K. Srinivasa Rao, Makkena Sreenu*, K.B.P. Raghavender and P.V.S. Kishore

ABSTRACT

Exploratory surgery is one of the diagnostic procedures followed to detect various abdominal disorders in bovine practice. Laparoscopy is a minimally invasive surgical technique using an endoscope inserted transabdominally to observe organs within the abdominal and pelvic cavities. The advantages of the use of laparoscopy-guided biopsy techniques are the direct visualization of the target organ and the selection of the exact biopsy site. In this way, obtaining biopsy specimens of the wrong organ is avoided, and possible hemorrhages are identified and controlled. The direct view of the target organ can provide additional information concerning the condition and eventually its prognosis.

Keyword: buffalo calves, *Bubalus bubalis*, liver, spleen, laparoscopic biopsy technique

MATERIALS AND METHODS

A total of twelve male buffalo calves aged about one and a half to two years presented to clinics were utilized to perform laparoscopy. Laparoscopy equipment along with accessories manufactured and supplied by Karlstorz (Germany) were used for this study. All the calves were kept

on fasting for 24 to 48 h prior to laparoscopy and they were bathed and dried before being allowed into operating room. The animals were administered with xylazine hydrochloride 0.05 mg/Kg body weight intra muscularly. Local infiltration of portal sites with 8 to 10 ml of 2% lignocaine hydrochloride (Xylocaine® Astra IDL Bangalore) was done in all the layers of the abdominal muscles for flank approach and subcutaneously for mid ventral approach prior to introduction of cannulas. The two portal sites selected to perform laparoscopy on the left side were at the middle and lower paralumbar fossa. For the mid-ventral approach, the portal sites selected were 2 inches lateral to mid-ventral line anterior to umbilicus. The biopsy specimens of the organs like the liver and spleen were collected under laparoscope guidance and after introducing a second cannula equidistant parallel or opposite to the first cannula introduced for a particular approach. To avoid injury to the abdominal structures, instrument cannulas are introduced under laparoscopic guidance and the instrument was pushed slowly into the cannula and entered the abdominal cavity. The jaws were kept closed until the instrument reached the required site if forceps or scissors were used. The collected tissue specimens were subjected to histopathological examination as per the method of Singh and Sulochana (1997) to study the tissue artifacts if any and to ascertain the suitability of the

Department of Veterinary Surgery and Radiology, NTR College of Veterinary Science, Gannavaram Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh, India,*E-mail: drmakkena@yahoo.co.in

yielded laparoscopic guided biopsy specimen for determining cellular architecture.

RESULTS AND DISCUSSION

Biopsy is a method aiding in the determination of a precise diagnosis and disease prognosis. Diagnostic evaluation of many different medical conditions can be assisted by obtaining biopsy samples from multiple abdominal organs. The sample collection has traditionally been performed several ways like fine-needle aspiration biopsy, percutaneous biopsy, biopsy under the guidance of ultrasonography, biopsy under endoscopic / otoscopic guidance, biopsy at the time of exploratory laparotomy (Mayhew, 2009). In cattle, the first reports on an organ biopsy by laparoscopy guidance involved the kidney (Naoi *et al.*, 1985) and the liver (Whitehair, 1998) while Klein *et al.* (2002) described an intestinal biopsy technique in calves and sheep.

Biopsy specimens of the spleen were collected using biopsy forceps through the instrument portal under the illumination of the left flank laparoscopy. The laparoscope was directed parallel to the spine cranially to identify the body of the spleen. The edge of the spleen was caught with the biopsy forceps, and a piece of about 1 mm size could be collected (Figure 1). There was haemorrhage from the cut surface of the spleen. A small swab of ear bud size was imbibed with adrenalin and placed on the bleeding area using forceps provided with the instrument for about 2 minutes to arrest the bleeding. Biopsy specimens of the spleen were collected in all the calves under the illumination of the left para lumbar laparoscopy. The technique is easy to perform and yielded an accurate sample size. Haemostasis was achieved

effectively with swabbing of adrenalin using biopsy forceps after collection of tissue for about two minutes. Meticulous care is needed while catching the edge of the spleen to crush the surface of the spleen to avoid multiple attempts (Figure 2).

In this technique, the laparoscope and instrument portals are created equidistance from the linea alba at xiphoid level on left and right sides to visualize the liver through midventral approach. Biopsy specimens of the liver were collected using biopsy forceps through instrument portal under the illumination of the midventral laparoscopy. The laparoscope was directed parallel to the right side cranially to identify the lobe of liver. The edge of the liver was caught with the biopsy forceps and the piece of about 1mm size could be collected. There was haemorrhage from cut surface of liver which was controlled by a swab of adrenalin as described earlier during the spleen biopsy. Biopsy specimens of the liver were collected using biopsy forceps through the instrument portal under the illumination of the mid ventral laparoscopy satisfactorily yielded in sample size. The search and visibility of the liver was good, and the edge of the liver was caught with the biopsy forceps on first instance in almost all the cases (Figures 3 and 4).

All the histological sections obtained from the laparoscopic guided biopsy specimens revealed a normal microscopic picture except for a few artifacts. The yielded sections of spleen revealed a dense connective tissue capsule from which connective tissue trabeculae extend deep in to the spleen interior and characterized by the presence of numerous aggregations of lymph nodules (white pulp) and surrounded by a diffuse cellular meshwork intermeshed with trabeculae (red pulp) along with arterial and venous structures (Figure 5). The sections of liver have shown the connective tissue from the liver hilus extends between the liver

lobes as indistinct interlobular septa dividing in to hepatic lobules. The interlobular septa had the branches of the portal vein, bile duct and hepatic artery (portal triad). In the centre of each lobule a central vein, cords of hepatic cells (the at periphery) and between hepatic cords, hepatic sinusoids are seen (Figure 6). Artifacts like separation of tissue, detachment of surface, vacuolization, cracking and mild congestion were observed at the edges of the tissue. (Figures 7-10).

Laparoscopic guided spleen and liver biopsy is a minimally invasive alternative to the biopsy methods by use of sharp cutting or grasping/shearing instruments. The technique selected depends on the surgeon's preference, stability of the animal and available equipment. Hidiroglou and Ivan (1993) conducted liver biopsies in sheep in sternal recumbency. Steve (2000) used laparoscopic techniques for biopsy collection from the spleen, liver, and kidney in horses and the hemostasis was achieved by using endoscopic bipolar cautery forceps.

As in open surgery, uncontrolled bleeding during laparoscopy is a major surgical pitfalls. A variety of techniques and instruments have been transferred from open surgery and adapted to the specific needs of laparoscopy to gain adequate haemostasis. Boure (2005) stated that control of intraoperative bleeding is most important in laparoscopic surgery and even a small amount of blood can obscure the laparoscopic surgical site because it absorbs the light and even cover the lens. The procedure adopted in the present i.e. swabbing of the cut surface with adrenalin following biopsy collection satisfactorily controlled the hemorrhage which might due to the vasoconstrictive property of the adrenalin.

Specimens obtained by laparoscopic guided biopsy techniques in the present study had minimal

distortion of tissue as evaluated microscopically and were considered an accurate representation of the organs and histological structures are similar to the observations of William and Linda (2000). All biopsy methods evaluated produced minimal immediate hemorrhage and resulted in adequate tissue samples for histological evaluation, (Vasanjee *et al.*, 2006). Damage was defined as any disruption of the normal cellular architecture at the incised margins and extent was determined by measuring the furthest edge of the damage perpendicular to the incised margin. Harmoinen *et al.* (2002) observed some inflammatory changes in sample collected through laparoscopic assisted biopsy around the biopsy sites

The biopsy forceps caused collateral damage, and two distinct forms of damage were apparent. Sharp cutting and grasping methods/instruments (biopsy punch, biopsy needle, ligature method, laparoscopic biopsy forceps) resulted in crushing of the tissue. The degree of crushing that occurs is a function of the instrument and handling of the tissues. For instance, an instrument like the laparoscopic biopsy forceps, where tissue is crushed and torn, would be expected to cause more collateral damage when performing a biopsy, the resultant tissue sample size is a function of the instrument used to obtain the biopsy. The biopsy needle, biopsy punch, and laparoscopic biopsy forceps are restricted in the amount of tissue obtained by the instrument size and design. Laparoscopy-guided biopsy of the liver and spleen yielded normal cellular architecture with minimum artifacts at the edges of the tissue collected due to the crushing effect of the forceps edges.



Figure 1. Note the biopsy forceps holding the splenic edge.



Figure 2. Hemorrhage from cut surface of the spleen following biopsy.



Figure 3. Collection of biopsy specimen from liver through ventral approach.



Figure 4. The hemorrhagic surface of the liver following the biopsy procedure.

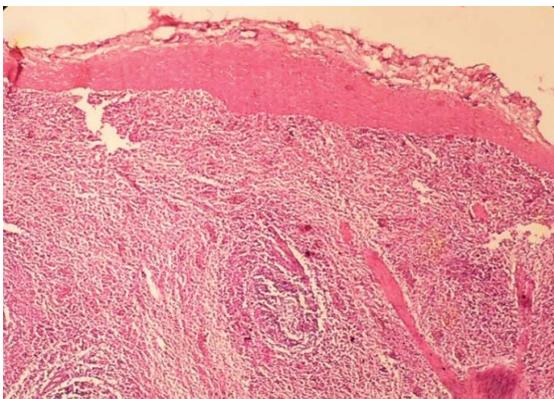


Figure 5. Cellular architecture of the spleen H&E 10 X.

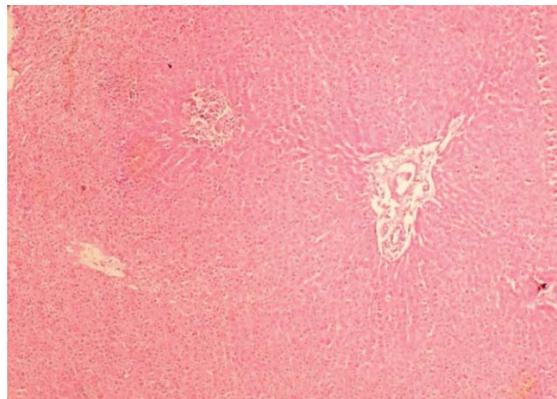


Figure 6. Cellular architecture of the liver H&E 10 X.

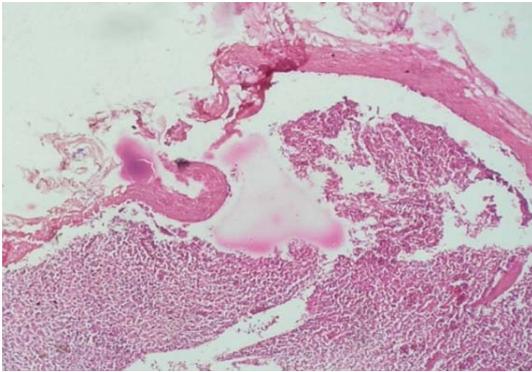


Figure 7. Cellular detachment and hyperemia– spleen. H&E 10 X.

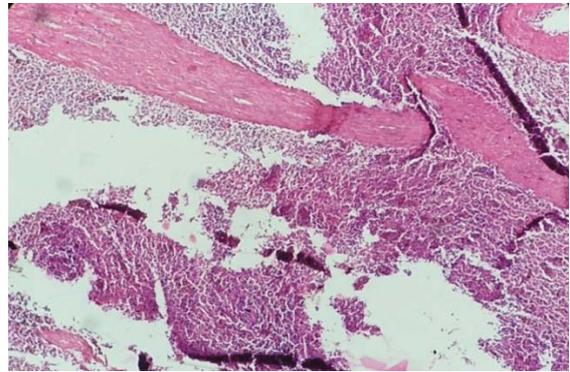


Figure 8. Folding and stain precipitate– -- spleen. H&E 10 X.

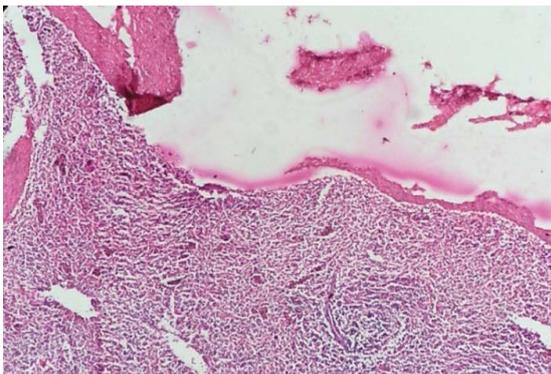


Figure 9. Detachment of the capsule - spleen. H&E 10 X.

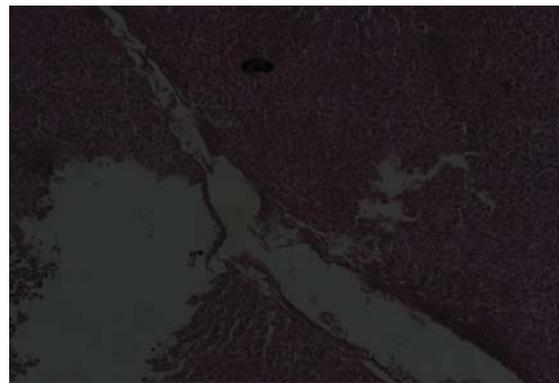


Figure 10. Cracking of the specimen- liver. H&E 10 X.

REFERENCES

144-147.

- Boure, L. 2005. General principles of laparoscopy. *Veterinary Clinics Food Animal Practice.*, **21**: 227-249.
- Harmoinen, J., S. Saari and M. Rininen. 2002. Evaluation of pancreatic forceps biopsy by laparoscopy in healthy beagles. *Vet. Ther.*, **3**: 31- 36.
- Hidioglou, M. and M. Ivan. 1993. Liver biopsy in sheep. *Vet. Res.*, **24**: 260-265.
- Klein, C., S. Franz, A. Leber, Z. Bago and W. Baumgartner. 2002. A new technique of laparoscopic biopsy sampling of the small intestine in calves and sheep [German]. *Wien Tierarztl Monatsschr.*, **89**: 291-301.
- Mayhew, P. 2009. Techniques for laparoscopic and laparoscopic assisted biopsy of abdominal organs. *Compendium: Continuing Education for Veterinarians*, 170-179.
- Naoi, M., E. Kokue and Takshakshi. 1985. Laparoscopic assisted serial biopsy of the bovine kidney. *Am. J. Vet. Res.*, **46**: 699-702
- Singh, U.B. and S. Sulochana. 1997. *A Practical Manual of Histopathological and Histochemical Techniques*, Kothari Publications, Bombay. pp. 154.
- Vasanjee, S. C., L.J. Bubenik, G. Hosgood and R. Bauer. 2006. Evaluation of hemorrhage, sample size and collateral damage for five hepatic biopsy methods in dogs. *Vet. Surg.*, **35**: 86-93.
- William, J.B. and M.B. Linda. 2000. General principles of histology, p. 1-8. *In Colour Atlas of Veterinary Histology*, 2nd ed. Lippincott Williams and Wilkins. USA.
- Whitehair, C.K., R.B. Dasilva and N.K. Anes. 1988. Live biopsy in cattle. *Bovine Practice.*, **23**: