Key Points

- Minimally Invasive Surgery (MIS) biopsy techniques can be performed under standing sedation and local anesthesia, in very ill patients and with reduced complication rate. Improved visualization represents the main advantage vs. other transcutaneous biopsy techniques.
- Biopsy sample size is also greatly amplified with MIS biopsy techniques, improving the number of analyses performed and the diagnostic potential of the procedure. Particularly for the lung, MIS biopsy techniques developed allow for large and safe sample collection.
- MIS full-thickness intestinal biopsy can be technically challenging; hybrid approaches should be considered when instrumentation and expertise is lacking.

What is MIS?

Surgical procedures performed completely under endoscopic guidance are regarded as MIS. Thoracoscopic and laparoscopic procedures are carried out by entering the thorax or the abdomen through small skin incisions. Natural Orifices Transluminal Endoscopic Surgery (NOTES) procedures are also considered as MIS procedures, giving access to the abdomen without any external incisions. They are carried out by entering the body through an anatomical opening (mouth, anus, urethra, vagina) and then through a body cavity (stomach, colon, bladder, vagina). Although at the early stage in Veterinary Medicine, NOTES procedures are gaining popularity in people and specific instrumentation has been developed.

Different biopsy techniques available in the thorax and abdomen

A biopsy is a surgical procedure defined as the sampling of tissue to determine the presence or the extent of a disease. Compared to fine needle aspiration or fluid collection, a tissue biopsy preserves the architecture of the tissue’s cells, which is a huge asset in the characterization of the disease. The harvested tissue is usually submitted for histopathological analysis. Additional tests are also commonly performed on tissue biopsies for clinical or research purposes (bacterial/fungal culture, virology, chemical analysis, DNA extraction, etc). When multiple analyses are necessary, the volume of tissue required increases significantly and the use of ultrasound-guided needle biopsies become obsolete (e.g. 18G Tru-Cut biopsy needle provides a sample of 0.8 mm diameter by 10-20 mm long, volume equivalent of 0.005-0.01 cm³).

To harvest bigger biopsy samples, open approach and MIS biopsy techniques are available. At the time abdominal/thoracic biopsies are needed to advance the diagnosis, the animals are commonly systemically and chronically affected and sometimes at the end-stage of a disease. Open approach biopsy techniques, commonly performed under general anesthesia, appear therefore less attractive in severely ill patients where healing potential is impaired. MIS biopsy can be performed under sedation and local anesthesia if general anesthesia is deemed too risky.
Benefits of MIS biopsy techniques

Beyond the fact that much bigger biopsy samples can be obtained, MIS biopsy techniques offer the serious advantage of direct visualization of the target organ, allowing selection of the exact biopsy site. To provide valuable information beyond the organ surface, laparoscopic ultrasonographic transducers have been developed. The real-time information provided by intra-operative ultrasonography has been shown to increase safety and accuracy of biopsy procedures among others. The preceding endoscopic exploration of the body cavity usually contributes to the diagnosis of the condition and prognosis. The development of post-biopsy complications such as hemorrhage or leakage from hollow organs can be immediately detected and addressed. Inadvertent biopsy of unintended organs is avoided unlike US-guided biopsy techniques where the risk is not negligible in certain situations.

Parenchymal/solid organs and masses – MIS biopsy techniques for small volume samples

Biopsy forceps are available in 10 mm diameter but a wider range of tip is available in 5 mm diameter with a working length varying from 35 to 45 cm. A variety of oval or “cup” style biopsy forceps (Blakesley, Manhes) can be used on the liver, spleen, pleura/lung and peritoneum, lymph nodes, and masses. The volume of a sample collected with a 5 mm cup biopsy forceps is 0.5 cm³, i.e. 50-100 times bigger than with an 18G Tru-Cut biopsy needle. “Punch” style biopsy forceps (Frangenheim) are more commonly used on the pancreas. As the pancreas is rarely a problem in horses compared to small animals, an oval biopsy forceps represents a more essential investment when economics comes into account. Teeth or spikes are available on some biopsy forceps jaws to better secure the piece of tissue harvested; the disadvantage is additional trauma to the harvested tissue. The presence of hemorrhage from the biopsy site can be stopped by application of direct pressure with the biopsy instrument. It is also worth considering biopsy forceps with a handle equipped with a monopolar electrosurgical connection in case additional hemostasis is required. Evaluation of the patient’s coagulation profile is advised before liver biopsy. Application of absorbable gelatin sponges (Gelfoam, Pfizer; Surgifoam, Ethicon) can be of benefit if the hemorrhage persists.

Although not ideal but in case biopsy forceps are not available, an alternative is to use a traumatic/aggressive grasping forceps and some laparoscopic scissors. Atraumatic grasping forceps, although attractive as they would create less tissue trauma, have a much weaker holding capability and several applications of the atraumatic forceps are likely to induce more damage to the tissue in the end. The risk of losing the sample in the peritoneal/pleural cavity or within the trocar lumen is higher with atraumatic grasping forceps.

Small spleen, liver and lung biopsies are technically easily harvested when using cup biopsy forceps. When widespread changes are noted, biopsies should ideally be taken from the organ edges. If a large amount of tissue is necessary, several biopsies can be collected sequentially. Only one instrument portal is usually necessary as these organs are more or less fixed to the body cavity by various supporting structures. The cup biopsy forceps is open and used to penetrate the spleen/liver capsule or visceral pleura. The forceps is kept closed for 30 seconds and then pulled away before being withdrawn from the body cavity. Liver capsule tears have been reported when removing the biopsy sample. Sharpening of the biopsy forceps jaws should be part of the routine maintenance of these instruments. Post-operative pneumothorax secondary to air leakage is possible following pulmonary tissue harvesting with cup biopsy forceps but the risk is limited due to the size of the created defect.

Cup biopsy forceps may not be ideal to obtain a kidney biopsy as the kidney is covered by various amount of perirenal fat. Therefore it is either advised to take several biopsies from the same hole and to go deeper each time, and to evaluate the tissue harvested to make sure this is not just fat. Tru-Cut-style biopsy or core needles can be used to avoid this problem but the size of the sample may be much smaller depending on the needle size.
For lymph node biopsy, an incision of the mesentery or the mediastinal layer with laparoscopic scissors is important to expose the lymph node. Then the cup biopsy forceps is introduced in the created window and the biopsy taken.

Incisional/core mass biopsies are performed using cup biopsy forceps. A portion of the lump or a suspicious area is removed. Choose firmer areas rather than softer areas where increased vascularization and tumor tissue necrosis could be present. For excisional mass biopsies, dissection with monopolar/bipolar electrosurgical instruments is advantageous over regular laparoscopic scissors as the risk of bleeding and tumor cells spreading is limited.

**Pulmonary tissue - MIS biopsy techniques for large volume samples**

Three thoracoscopic techniques have been evaluated in standing heaves-affected horses in the last decade. Following a routine thoracoscopic approach, a minimum of 2 instrumental portals are necessary whichever biopsy technique is selected. The caudal edge of the lung is the target. Keep a safe distance (> 5cm) from the suspensory pulmonary ligament which is located in the dorsocaudal corner of the lung. To the author’s knowledge, there is no reported technique for large lung biopsy harvested in the middle of the equine lung field.

**Ligating loops**

More technically difficult but certainly the most cost effective, pre-tied USP 0 Polysorb ligating loops (Surgitite, Covidien) have proven to be fairly efficient to harvest large and excellent quality lung biopsies (surface ≥ 3 cm² for 75% of the samples collected; 1-3 cm² for 25% of the samples). A mildly traumatic grasping forceps is passed through the ligating loop and the caudodorsal tip of the lung is grasped, stretched slightly to allow the loop to engage the lung tissue. Then the loop is progressively tightened using the included knot pusher. The excess of suture material is cut with laparoscopic scissors and then the biopsy is released. It is important to leave enough tissue (> 5mm) above the suture to avoid ligature slippage, which is the most common complication with this technique (27%). Evaluation of the biopsy site is therefore advised to make sure the ligature is still in place at the end of the procedure. In case the ligature has slipped, an additional ligating loop can be re-placed to seal the defect. Some Endo-GIA staplers (total length 45 mm; staple size 4.1 mm; Covidien) are good back-up instruments if additional pre-tied ligatures cannot be properly secured. This technique is not recommended on very fibrotic lungs as the poor deformability of the tissue prevents proper ligature placement.

**Vessel-sealing and dividing device (Ligasure, Covidien)**

Large and excellent quality lung biopsies could be harvested with the Ligasure device (surface ≥ 3 cm² for 95% of the samples collected). A distance of 2.48 ± 1.06 mm from the biopsy margins was unreadable because of the thermal damage generated by the activation and energy transfer from the vessel-sealing device. The complication specifically related to this technique was an inadequate seal noted at the biopsy margin in 6% of the samples. Careful evaluation of the biopsy site is therefore advised after the biopsy release. In this study one heaves-affected horse (in severe crisis at the time of surgery) suddenly died 5 days post-surgery, presumably from a tension pneumothorax. Increasing or persistent post-operative pneumothorax may indicate an air leak from the biopsy site and placement of a thoracostomy tube connected to a Heimlich valve is strongly advised. Although technically less challenging and quicker than the ligating loop technique, the operator needs to be careful with portal placement as the tip of the Ligasure is not articulated. It is therefore easy to aim too dorsal and to rapidly engage the thick part of the lung. An effort should be made to stay as parallel to the lung edge as possible so as to avoid this problem.
Wedge lung resection with Endo-GIA stapler

Certainly the less cost effective technique, large (24 cm³) and excellent quality lung biopsies could be harvested with an Endo-GIA stapler (total length 45 mm, staple size 4.1 mm; Covidien). The edge of the lung is grasped, the stapler applied across it and then fired. Trocar repositioning was necessary in 2/10 horses to improve tissue handling. Specific complications associated with this biopsy technique were not reported. The largest stapler is recommended to limit the cost associated with the need for more than one staple line.

Full-thickness intestinal samples - MIS biopsy techniques

MIS full-thickness intestinal biopsies represent a bigger challenge for the operator as the gut is relatively mobile, the risk of lumen reduction and the contamination of the abdominal cavity are present. Two techniques have been evaluated on normal standing horses.

Two-step full-thickness technique using intracorporal suturing + knot substitute placement

The right flank was approached and full-thickness biopsies (7-10 mm long) from relatively fixed parts of the equine gut (descending duodenum and caecum) were harvested. The technique consisted of a one-layer closure using intracorporal suturing and knot substitute placement. The first step of the procedure consisted of harvesting a partial thickness piece of intestine corresponding to the seromuscular layer using laparoscopic scissors and a Kelly forceps. Once the seromuscular biopsy was removed, the partial thickness incision was partially closed using a Lembert pattern with a USP 3-0 resorbable suture material. A PDS knot substitute (Lapra-Ty, Ethicon) was placed at the start of the suture line. The submucosa and mucosa were periodically everted through the partially closed incision. Once the suturing was almost completed, the second step of the biopsy procedure was completed and submucosa/mucosa were excised with scissors. The biopsy was removed, the last stitch of the continuous incision was made and the suture tightened. A second knot substitute was placed at the end of the suture line.

None of the horses (n=4) developed duodenal impaction or septic peritonitis. The biopsy site was flushed and an abdominal lavage performed at the end of the procedure as part of the experimental protocol. The biopsies collected were considered to be of good quality. Although an alternative to intracorporal knot tightening was used in this study, the level of laparoscopic skills required to use this technique is high and should be reserved to laparoscopic surgeons with advanced training.

Full thickness technique using Endo-GIA

The right flank was approached and full-thickness biopsies (32 mm long, 14 mm thick) were harvested from the equine descending duodenum and jejunum using an Endo-GIA stapler (total length 45 mm, staple size 4.1 mm; Covidien). The technique consisted of grasping and applying tension on the antimesenteric border of the selected portion of small intestine. The stapler was then applied and a V-shaped biopsy was collected.

In 1 biopsy out of 52, the stapler failed to engage properly through the full thickness of the intestinal wall. A suture layer was placed on top of the staple line with the Endo-Stitch device. As for the previous technique, a post-operative non-septic inflammatory reaction was noted in the peritoneal cavity but no post-surgical complications were noted. The biopsies collected were considered to be of good quality. Second-look laparoscopy was performed on 38 biopsies; 4 biopsy sites presented omental adhesions that were clinically silent. Although the use of an automated stapler decreased the level of skills required to perform full-thickness intestinal biopsies, the surgeon needs to be ready to convert the procedure into an open approach if a serious problem occurs and intracorporal suturing is not mastered.
Hybrid procedures

The cost and the level of training necessary to perform MIS full-thickness intestinal biopsies may force some surgeons to consider “hybrid” solutions. The exploration is performed by laparoscopy and the part of the intestine to be biopsied is exteriorized through a small flank laparotomy incision. The instrumental portal is actually enlarged to allow the piece of intestine to be milked through the wound. The biopsy is performed outside of the abdominal cavity using routine hand-sewn technique. Although not considered as a true MIS as part of the procedure is not completed under endoscopic guidance, these hybrid procedures are still less invasive than open approaches.

Contraindications and disadvantages

There are no real contraindications specific to MIS biopsy technique but the general contraindications related to laparoscopic and thoracoscopic procedures apply. The cost of certain MIS biopsy techniques can be prohibitive, particularly in cases where a poor prognosis is suspected. MIS involves different instrumentation, visual and tactile skills than traditional open surgeries so the cost of equipment and proper surgical training needs to be considered.