

# Standard Operating Procedures - sheep Fistulation of gastro-intestinal tract

Ref Code: SHE09

## Category

Research - 5

## Objective

To prepare sheep so that relevant samples may be collected or infusions made, under physiological conditions.

## Details of Procedure

Only sheep greater than three months of age should be used.

Animals must be anaesthetised for the procedure. Sodium pentobarbitone (60 mg/mL; 1 grain/mL), is used at approximately 0.30 mL/kg body weight to induce general anaesthesia. Half is given rapidly as a single injection and the remainder slowly after two to three minutes until the desired depth of anaesthesia is reached. This will provide surgical anaesthesia for 20- 30 minutes for most animals. The surgical procedure should take no longer than 15-20 minutes to complete (depending on the procedure). In addition, a local anaesthetic is given at the site of surgery - approximately 5-8 ml of 2% xylocaine is used.

The side of the sheep is clipped and scrubbed down with Iovone(R), followed by chlorhexidine gluconate (0.5% w/v) in 70% alcohol.

## General Procedure

The general procedure for inserting a cannula into the gastrointestinal tract (that is, creating a cannulated fistula) is as follows.

1. The appropriate part of the tract is located and precautions are taken to avoid contaminating surrounding tissues with digesta. Included in these precautions is the stuffing of a swab or bung into the lumen of the barrel of the cannula to prevent escape of digesta.
2. An elliptical purse string suture is placed in the wall of the gut, passing into the lumen so that it penetrates the tough submucosal layer. For a cannula in the small intestine, this suture is placed in the antimesenteric side of the intestine. A rounder suture may be placed in viscera other than the small intestine, and if the cannula has a circular internal flange, the length should be slightly greater than the diameter of the flange.
3. An incision is made inside the purse string suture. This is readily achieved by pushing one point of a pair of sharp-pointed scissors through the wall and cutting. Do not cut the suture.
4. The internal flange of the cannula is inserted into the visceral lumen. This is facilitated with a ruminal or abomasal cannula if an assistant holds up the wall with one or two pairs of Allis forceps. Holding up the wall will reduce the risk of spilling digesta. For ruminal cannulation of sheep, a Jarret or a Hogan (size A-G) cannula is commonly used; for cattle, Hogan (size A-H) or the inflatable types are appropriate.
5. The suture is tightened and tied. Another suture may be required if the incision is not completely closed. The insertion of a second suture around the barrel is rarely needed and is contraindicated for the small intestine as its lumen may be constricted. It is often recommended that the cut edge of mucosa be inverted but this does not seem to be necessary.
6. The peritoneal flange (if used) is then inserted, the omentum is located, an incision is made through the omentum and the edges are draped over the barrel.
7. The barrel is exteriorised, either through the laparotomy incision by closing the muscles and then the skin around the barrel, or by making a separate stab wound, using a pair of tissue scissors. The points of the scissors are forced open to make a hole

of sufficient size to take the barrel of cannula. The barrel is passed through, either by guiding it with a wire attached to a cone in the end of the barrel or by passing a pair of forceps through the hole and grasping the barrel. The cannula should be pushed from below rather than from above.

8. The final step is to add the external retaining flange. This must be firm enough to hold the cannula against the abdominal wall to allow adhesions to form rapidly, thus obviating the need for stay sutures. Excess pressure on the tissues between the flanges delays healing. Once adhesions have formed around the fistula between the peritoneal linings of the visceral and abdominal walls, the peritoneal cavity is effectively sealed and the risk of peritonitis correspondingly reduced.

### 1. Oesophageal Fistulation

Oesophageal fistulation has been established in sheep for the purpose of obtaining samples of the forage ingested by the grazing animal. The forage samples may be used for botanical and chemical analysis and for in vitro digestion.

The skin incision is performed in the mid-cervical region on the left side of the neck. The procedure then is to expose the oesophagus and suture the mucosa and muscular tunic to the skin. Closure of the wound is completed by inserting a rigid cannula, T-plug (Macaam Rubber).

The operation is facilitated by the use of a metal rod covered at one end with rubber (probang) which is used to elevate the oesophagus to a suitable site. This approach alleviates the task of unwanted dissection. The incision is extended and sutured in step-wise manner until the fistula is the required size for the insertion of the well lubricated T-plug. The probang is then removed.

As little trauma is done to the tissue, recovery is rapid. Prospective surgeons are referred to references and an excellent description is found in Hecker (1974).

### 2. Duodenal Cannulation

A single cannula may be inserted into any part of the small or large intestine. Digesta will flow readily from a cannula in the small intestine and may be sampled through a cannula in the caecum or the broad colon. After the end of the broad colon, digesta is of too firm consistency for sampling and a cannula can only be used for the introduction of substances in the lumen.

Probably the easiest part of the intestine to cannulate is the start of the duodenum. Only a short skin incision, about 5 cm, is needed and this is made immediately behind the ventral end of the second last rib. A hole is made in the muscles and peritoneum by blunt dissection and the duodenum is located by passing a finger through the laparotomy incision and feeling for it either under the incision or under the last rib. The duodenum here is the only part of the intestine not covered by omentum. A sufficient length is hooked out through the incision to enable a purse string suture to be placed in the wall and a cannula to be inserted through an incision inside the suture. The barrel of the cannula is best exteriorised by closing the laparotomy around it, although it is possible to pass the barrel out through a stab wound made through the rib cage.

Larger incisions behind the ventral end of the last right rib sometimes develop hernial swelling. These swellings led to the development of a technique in which the whole operation of duodenal cannulation was performed through a resected part of the eleventh rib. These swellings are unlikely to develop if the duodenum is cannulated through the small incision described in the above paragraph and therefore this transcostal approach is best reserved for sheep in which previous surgery has made a paracostal incision undesirable.

A right paravertebral laparotomy provides easy access to the part of the duodenum immediately after the entrance of the common bile and pancreatic ducts. There is little difficulty in inserting cannulae into the jejunum or the ileum once these parts are located, and several small intestinal cannulae may be exteriorised through the right flank while one or two may also be exteriorised in the dorsal part of the left flank.

Numerous types of cannulae have been used for the intestine but most are similar in that they have a straight barrel joined to a gutter-shaped internal flange to form a "T". Certain types of plastic disposable syringes make satisfactory cannulae for the intestine if the barrel is cut 3-4 cm from the finger holds and these are used as internal flanges. Syringes should be made from one of the less brittle plastics.

### 3. Rumen Cannulation

This may be carried out to examine rumen metabolites and microflora; monitor the activity of slow release devices; or obtain inoculant for in vitro digestibility assays.

As an alternative to general anaesthesia, the subject can be sedated with xylazine (Rompun(R)) and the surgical site anaesthetised either by a) a paravertebral block at T13, L1, L2 and L3 with 8-10 ml 2% lignocaine or b) an inverted "L" block caudal to the last rib and ventral to the edge of the lateral processes of the lumbar vertebrae with 8-10 ml 2% lignocaine.

#### Variation for Ruminal Cannulation

Following previously described pre-operative and anaesthetic procedures, the following modifications may be used:

1. Locate the rumen via an incision below the proposed site of the cannula. The cannula should be fixed below the ends of the transverse processes on the left flank. Hecker (1974) refers to this area as the anterodorsal portion of the flank. Identify a portion of the tract that would lie close to the proposed fistula. It is possible to locate this position by using a cattle rumen injector gun.
2. Insert a double elliptical string suture using 1/0-size silk suture with a diameter the size of the neck of the cannula.
3. Plug the cannula with either cottonwool or a rubber bung to stop digesta escaping when fixing the cannula into position.
4. Make a blunt dissection into the rumen inside the purse string suture and by inverting the flange on the cannula and securing the rumen mucosa with forceps, push the cannula into position. Tie off both sutures and push any exposed mucosa under the suture, check for leakages.
5. The neck of the cannula is exteriorised via a stab wound made anterior to the current incision. Try to minimise the size of the stab wound, making the neck of the cannula a tight fit.
6. Once the incision is closed and the cannula positioned, place backing plate over the neck of the cannula. This will secure it from falling back into the rumen and will enhance the adhesion of the rumen mucosa to the visceral wall. This plate should not be made too tight.

In pregnant ewes, and where anaesthesia is deleterious to the animal or experiment, a rapid method devised by Hecker (1974) is suggested. This involves a two-stage process where the rumen is exposed and clamped off in the conscious animal and after a few days this portion sloughs off and a plug is inserted into the fistula.

#### 4. Abomasal Cannulation

The surgical approach to cannulation of the abomasum is well documented. This technique, as commonly performed, has several inherent defects. Foremost amongst these is the inability to keep the cannula in place as a result of its protrusion from the lower abdominal wall immediately beneath the costal arch. A change in the position of the cannulae is desirable to ensure more stable placement and, thus, add further insurance that any research project using cannulated animals would not be interrupted prior to completion. Any site on that portion of the body wall overlying the abomasum, other than the classic one, immediately becomes involved with the complexities associated with the rib cage.

The cannula may be sited in the area of the ninth rib. To allow this, a second incision is made approximately 5 cm distal and parallel to the costo-chondral junction. Through this latter incision the abomasum is brought to the outside and the cannula fixed in place. At the proximal site an incision is made into the abdominal cavity sufficient to allow for placement of the abomasal fixture. The organ, with its cannula in place, is then brought out the more proximal incision and secured.

1. Position the cannula at the ninth rib. The initial incision to provide the placement of the cannula is made distal but parallel to the last rib on the right side of the sheep.
2. The abomasum is located (use pyloric sphincter as a landmark) and the cannula is fixed into position. The procedure of fixing the cannula to the abomasum is similar to that described for the rumen, see also Hecker (1974). The site for the placement of the cannula into the abomasum is preferred more proximal to the cardiac sphincter to reduce interference with pyloric function.
3. The same stab wound technique as employed in the rumen cannulation is used to exteriorise the cannula through the body wall at the ninth rib.

#### Care and Maintenance of Cannulae

Most gastrointestinal cannulae cause little trouble and require little maintenance. The external flange should be removed occasionally so that the wool underneath can be clipped. However, if the fistula is slack and digesta leak around the cannula, it is better not to clip regularly.

### Occasional Problems

1. Most fistulae will close when their cannulae are removed or catch on an obstruction and pull out. This closure can be rapid, and a fistula may contract to half its normal diameter within a few hours, and can close completely within a few days. Therefore a cannula that has pulled out should be replaced as soon as its loss is noticed. If the internal flange will not enter the fistula, the animal should be anaesthetised and the fistula dilated by pressure with large forceps.
2. Pressure on one side of the base of a cannula may cause erosion of tissues with the result that one edge of the base breaks through the skin and the cannula comes away from the fistula. Little can be done to repair such damage and it may be necessary to insert a larger flexible cannula.
3. A cannula may slip into the lumen of the gut if the external flange comes off. A rumen cannula will normally come to lie at the bottom of the reticulum and can be readily recovered.

### Infusion into the Gastrointestinal Tract

Only a small diameter cannula is needed when solutions are to be infused into the gastrointestinal tract. A satisfactory cannula can be made from a length of silicone rubber or other plastic tubing, which is passed through a hole in a square of Dacron mesh and glued with silicone rubber adhesive or epoxy resin. This end of the cannula is inserted into the visceral lumen through an incision inside a purse string suture, which is tied, and the mesh is anchored to the peritoneal wall of the viscus by four single sutures. This cannula can be inserted into any part of the tract and is usually very successful. The cannula is best occluded with a stilette when not in use.

## **Contraindications for use of procedure**

Do not perform on pregnant animals unless absolutely necessary for subsequent experimentation.

## **Drugs, chemicals or biological agents:**

Sodium pentobarbitone, xylocaine

Impact of procedure on wellbeing of animals:

Normal physiological status is attained in cannulated animals.

## **Reuse and repeated use**

Animals should only undergo surgery for fistulation once. With care, however, a fistulated animal may be used for a number of different experiments over time.

## **Care of animal(s) during/after the procedure:**

A suitable course of parenteral antibiotic (e.g. Terramycin Long-acting) is given whenever major surgery is undertaken. Animals should be allowed to graze succulent pasture during the initial period after surgery.

The sheep should be inspected daily and the cannula cleaned as necessary. Any loss of body weight or inappetance should be noted. Due to the social behaviour of sheep, they are best kept in a small flock in a protected paddock for observation, and any that stray away from the flock should be given special attention. Posture is also a useful indication of potential problems.

An animal with an oesophageal fistula may lose its appetite. This tends to occur more frequently if a sheep is given a coarse roughage diet. A change of diet is the best cure, either to a pelleted ration or by turning the animal onto green pasture. Salt should always be available for fistulated sheep because of salivary losses. Although it has been suggested that sheep should not be used for several weeks after oesophageal fistulation, they may be used much sooner if healing is uneventful. Lambs have been used within six to ten days and wethers within 21 days of fistulation.

An occasional problem is enlargement of the fistula with subsequent leakage or loss of the plug or cannula. The most satisfactory remedy is to use a larger plug.

## Pain relief measures

General and local anaesthesia.

## Qualifications, experience or training necessary to perform this procedure:

Sound knowledge of anatomy, aseptic technique and anaesthesia. The person performing the surgery must be under the supervision of a veterinarian.

## Reference

"Compendium of approved procedures". Division of animal production. C S I R O Division of Animal Health Armidale, 2350 pp 42-49 1995.

Hecker, J.F., (1974). Surgery on the gastro-intestinal tract. In Experimental Surgery on Small Ruminants.