May 02, 2024

Protocol for surgical implantation of chronic cecal cannula in pigs

DOI
dx.doi.org/10.17504/protocols.io.8epv5jwx5l1b/v1

Muriel Larauche[1], Karim Atmani[1], Yushan Wang[1], Zach Weitzner[1], Sandra Duarte-Vogel[1], Wentai Liu[1], Mulugeta Million[1], Justin Wagner[2]

[1]University of California, Los Angeles; [2]UCLA David Geffen School of Medicine

SPARC
Tech. support email: **info@neuinfo.org**

Muriel Larauche
University of California, Los Angeles

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.8epv5jwx5l1b/v1

Protocol Citation: Muriel Larauche, Karim Atmani, Yushan Wang, Zach Weitzner, Sandra Duarte-Vogel, Wentai Liu, Mulugeta Million, Justin Wagner 2024. Protocol for surgical implantation of chronic cecal cannula in pigs . **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5jwx5l1b/v1

Protocol status: Working
We use this protocol and it's working

Created: May 26, 2023

Last Modified: May 02, 2024

Protocol Integer ID: 82529

Keywords: cecum, proximal colon, chronic implantation, cannulation, surgery

Funders Acknowledgement:
SPARC NIH
Grant ID: OT2OD024899

Abstract

Gastroduodenal and ileal cannulas in pigs are commonly used to evaluate food digestibility and nutrition research. However accessing the motility of the proximal colon for long term in conscious pigs remains a challenge. In this protocol, we describe the surgical implantation of a cecal cannula in Yucatan minipigs, together with details to design/3D-print a biocompatible cecal cannula that allows direct access to the cecum/proximal colon of pigs and the maintenance procedure. The cannula is very well tolerated by the animals for long-term use. The model enables not only a reliable longitudinal chronic colon function measurements (probes inserted through the cannula) to study gut motility in health and diseases but also for luminal sensing and stimulation using stationary or non-stationary wireless smart pills.

Materials

- Four months old (18-22 kg) male (castrated at 7 days of age) and female Yucatan minipigs (S&S farms, Ramona, CA)
- Low residue food (Hill's Prescription Diet i/d Dry Dog Food, #2684397 or LabDiet Advanced Protocol 0043639 (594K))
- Liquid dietary supplement (Impact Advanced Recovery®, Nestlé Health Science or Ensure Surgery Vanilla flavor, Abbott Laboratories)
- 8 oz of Miralax (Polyethylene Glycol 3350, Bayer US) mixed in 32 oz of Gatorade or Pedialyte
- Dulcolax (Bisacodyl, 5 mg, Sanofi)
- Oral gabapentin at 5 mg/Kg
- 20 mg of omeprazole
- Tiletamine and zolazepam at 6 mg/Kg, IM
- Isoflurane (2-4%)
- Alcohol/chlorhexidine solution
- IV catheters
- Meloxicam (0.3 mg/kg IM)
- Hydromorphone 0.1 mg/Kg IV
- Famotidine at 1 mg/Kg IV
- Maropitant (Cerenia, Zoetis) at 1 mg/Kg IV
- Buprenorphine 0.02mg/kg IV/IM
- Ethiqa SR 0.2 mg/kg
- Meloxicam 0.3mg/kg SID (PO/or IM)
- Excede 5mg/kg IM
- Cerenia 2mg/kg SID PO
- Omeprazole 20mg/day PO
- Long acting tulathromycin (Draxxin, Zoetis) at 2.5 mg/Kg IM
- Long acting Ceftiofur (Excede, Pfizer) at 5 mg/Kg IM
- Cefazolin at 30 mg/Kg IV
- Lactated Ringer's solution (USP, Preservative-Free, Baxter Henry Schein Animal Health Catalog #059380) at 5-10 ml/kg/h IV
- 0.25% bupivacaine at 0.6 mL/Kg per site (2-4 sites) (Marcaine or bupivacaine hydrochloride, Henry Schein Animal Health)
- Lidocaine 2 mg/Kg
- Marcaine 2 mg/Kg
- Heating pad (32°C)
- Heparinized saline
- Loban (Steri-Drape, 3M)
- Autoclaved surgical tools
- Disposable scalpel
- Monopolar electrocautery device
- 0-looped PDS on CTX needle (PDP990G, Ethicon)
- Absorbable suture (2-0 PDS on SH needle (7317H, Ethicon)
- Skin glue (Vetbond Tissue Adhesive, 3M Animal Care)
- Chlorhexidine solution (Chlorhexidine 2% Solution, VetOne, Catalog #1054488)

- Zinc oxide ointment (Desitin, 40% zinc oxide, Johnson & Johnson Pediatrics)

Protocol materials

Meloxidyl **Zoetis Catalog #**NDC 11695-6966-2 In 2 steps

LabDiet Advanced Protocol 0043639 **LabDiet Catalog #**594K Step 2

Midazolam HCl Injection, 50mg/10mL (5mg/mL), C4 **Henry Schein Animal Health Catalog #**067695 Step 4

Famotidine USP 20 mg **BluePoint Laboratories Catalog #**NDC 68001-397-00 In 2 steps

Cefazolin for Injection, USP **Pfizer (Hospira)** Step 2.1

Telazol **Zoetis** Step 2.1

Cecal cannula ABS-M30i material, biocompatible **Stratasys Direct Manufacturing** Step 2.1

Impact Advance Recovery **Nestle Health Science Catalog #**00043900943114 Step 2

Ensure Surgery Vanilla flavor **Abbott** Step 2

Cerenia **Zoetis** In 2 steps

Lidocaine 2% **VetOne Catalog #**NDC 13985-222-04 Step 2.1

Ioban 2 23x23 Sterile Surgical Drape Non-Fenestrated **Henry Schein Animal Health Catalog #**7774142 In 2 steps

Desitin **Johnson & Johnson Pediatrics** Step 3

IsoThesia (Isoflurane) Solution **Henry Schein Animal Health Catalog #**029405 In 3 steps

Chlorhexidine 2% solution **VetOne Catalog #**11-09007 In 3 steps

Draxxin **Zoetis** Step 2.1

Dulcolax (Bisacodyl 5 mg) **Sanofi** Step 2

EloxiJect (Meloxicam) Injection, 5mg/mL **Henry Schein Animal Health Catalog #**049755 Step 4

3-0 polydioxanone suture on SH needle **Ethicon Catalog #**J316H Step 2.1

Ethiqa XR (1.3 mg/ml) **Fidelis Pharmaceuticals Catalog #**NDC 86084-100-30 Step 2.2

Miralax (Polyethylene Glycol 3350) **Bayer** Step 2

Ketamine HCl Injection, 200mg/20mL (10mg/mL), C3N **Henry Schein Animal Health Catalog #**068317 Step 4

2-0 PDS on SH needle **Ethicon Catalog #**7317H Step 2.1

Gabapentin (oral solution, 250 mg/5 ml) **Amneal Pharmaceuticals LLC Catalog #**NDC 65162-698-90 Step 2.2

i/d Dry Dog Food **Hill's Pet Nutrition Catalog #**2684397 In 2 steps

0-looped PDS on CTX needle **Ethicon Catalog #**PDP990G Step 2.1

Hydromorphone Hydrochloride Injection, USP **Teva Pharmaceuticals USA, Inc Catalog #**NDC 0703-0113-03 In 2 steps

Heparin Sodium Injection USP. 1000U/mL **Pfizer (Hospira) Catalog #**NDC 0409-2720-02 Step 2.1

Lactated Ringers Injection, USP, Preservative-Free, Baxter **Henry Schein Animal Health Catalog #**059380

In 2 steps

Heparin Sodium Injection, USP, 10,000 unit/mL **Henry Schein Animal Health Catalog #**067792 Step 4

Laboratory Mini-Pig Grower Diet 5081 **LabDiet Catalog #**0001339 Step 1

Vetbond Tissue Adhesive **3M corporation Catalog #**1469SB Step 2.1

Marcaine Injection 0.5% **Henry Schein Animal Health Catalog #**6312615 Step 2.1

Prilosec OTC (omeprazole 20 mg) **Procter and Gamble Catalog #**NDC 37000-455-02 Step 2.2

Sodium Chloride Injection, USP, Preservative-Free, 0.9%, Baxter **Henry Schein Animal Health Catalog #**059382

In 2 steps

Yucatan minipig **S&S Farms** Step 1

Excede **Zoetis** In 2 steps

Animals

1 Four months old (18-22 kg) male, castrated at 7 days of age, and female Yucatan minipigs (S&S farms, Ramona, CA) , were singly housed in pens (either bedding or grate floor depending on housing availabilities - 2 pigs/pen, 42ft^2) in an environmentally controlled room (lights on/off 6AM/6PM, 61-81°F) under SPF conditions.

Yucatan minipig **S&S Farms**

All pigs were offered ad libitum access to diet (Laboratory Mini-Pig Grower Diet 5081, LabDiet) and filtered tap water.

Laboratory Mini-Pig Grower Diet 5081 **LabDiet Catalog #**0001339

All husbandry practices and procedures conformed to the Guide for the Care and Use of Laboratory Animals (8th edition), the Animal Welfare Act and were reviewed and approved by the UCLA Institutional Animal Care and Use Committee.

Surgical cannula implantation through laparotomy

2 **Pre-operative diet regimen for intestine preparation**

Five days prior to surgery, the animals were started on a low residue diet consisting of 2 cups of low residue food (Hill's Prescription Diet i/d Dry Dog Food or LabDiet Advanced Protocol 0043639 (594K)) plus one bottle of a liquid dietary supplement (Impact Advanced Recovery®, Nestlé Health Science or Enure Surgery Vanilla Flavor, Abbott Laboratories) twice daily.

i/d Dry Dog Food **Hill's Pet Nutrition Catalog #**2684397

LabDiet Advanced Protocol 0043639 **LabDiet Catalog #**594K

Impact Advance Recovery **Nestle Health Science Catalog #**00043900943114

Ensure Surgery Vanilla flavor **Abbott**

Two days prior to the surgical procedure, 8 oz of Miralax (Polyethylene Glycol 3350, Bayer US) mixed in 32 oz of Gatorade or Pedialyte plus 2 pills of Dulcolax (Sanofi) was administered once a day for 2 days. Oral Gabapentin at 5 mg/kg and 20 mg of omeprazole were administered the day before surgery.

Miralax (Polyethylene Glycol 3350) **Bayer**

Dulcolax (Bisacodyl 5 mg) **Sanofi**

2.1 Surgery

Pigs were fasted for at least 12h prior to surgery with free access to water.

Anesthesia was induced with Telazol (Tiletamine/zolazepam, 6 mg/kg, IM) , followed by isoflurane delivery initially via face mask and later via endotracheal tube. Both ears were cleaned with 3 alternating scrubs of alcohol/chlorhexidine solution and IV catheters were inserted and secured into the lateral or medial veins, for administration of medications and fluids.

- Telazol **Zoetis**
- IsoThesia (Isoflurane) Solution **Henry Schein Animal Health Catalog #**029405
- Chlorhexidine 2% solution **VetOne Catalog #**11-09007

For pre-operative analgesia animals received Meloxidyl (meloxicam, 0.3 mg/kg, IM), and hydromorphone (0.1 mg/Kg, IV).

- Meloxidyl **Zoetis Catalog #**NDC 11695-6966-2
- Hydromorphone Hydrochloride Injection, USP **Teva Pharmaceuticals USA, Inc Catalog #**NDC 0703-0113-03

Other medications administered pre-op include: Famotidine at 1 mg/Kg IV, Cerenia (Maropitant citrate, Zoetis) at 1 mg/Kg IV, long acting Draxxin (tulathromycin, Zoetis)at 2.5 mg/Kg IM, long acting Excede (ceftiofur, Pfizer) at 5 mg/Kg IM, and Cefazolin at 30 mg/Kg IV.

- Famotidine USP 20 mg **BluePoint Laboratories Catalog #**NDC 68001-397-00
- Cerenia **Zoetis**
- Excede **Zoetis**
- Draxxin **Zoetis**
- Cefazolin for Injection, USP **Pfizer (Hospira)**

Intra-operatively, a surgical plane of anesthesia was maintained with isoflurane (2-4%) delivered via endotracheal tube. The animals were also mechanically ventilated with a tidal volume of 10-20 mL/Kg and a respiratory rate of 10-16 breaths per minute.

- IsoThesia (Isoflurane) Solution **Henry Schein Animal Health Catalog #**029405

Lactated Ringer's solution was administered at 5-10 ml/kg/h IV throughout the surgery.

Lactated Ringers Injection, USP, Preservative-Free, Baxter **Henry Schein Animal Health Catalog #**059380

In some experiments, prior to incision, 0.25% bupivacaine at 0.6 mL/Kg per site (2-4 sites) (Marcaine or bupivacaine hydrochloride, Henry Schein Animal Health) was injected in the transverse abdominalis plane using ultrasound to guide the regional block. Otherwise, lidocaine 2 mg/Kg was injected subcutaneously at the incision sites pre operatively followed by marcaine 2 mg/Kg at closure.

Marcaine Injection 0.5% **Henry Schein Animal Health Catalog #**6312615

Lidocaine 2% **VetOne Catalog #**NDC 13985-222-04

During the surgical procedure, pigs were positioned on a heating pad (32°C) in supine position. The right medial thigh was also cleaned and the right femoral artery was catheterized to monitor blood pressure during the procedure. Heparinized saline was used to flush the line preventing clotting.

Sodium Chloride Injection, USP, Preservative-Free, 0.9%, Baxter **Henry Schein Animal Health Catalog #**059382

Heparin Sodium Injection USP. 1000U/mL **Pfizer (Hospira) Catalog #**NDC 0409-2720-02

The left and ventral abdomen side of the animals was scrubbed with Chlorhexidine using a pre-packed surgical brush. Hair in this area was clipped and the skin prepared aseptically alternating chlorhexidine solution and alcohol. Cannula exit was marked at the dorsal flank area with a sterile marker (between the last rib and the antero-dorsal region of the flexed stifle joint).

Chlorhexidine 2% solution **VetOne Catalog #**11-09007

The surgical site was draped with Ioban (Steri-Drape, 3M). Sterile drapes were placed on top of the Ioban-covered surface with an open small widow/surgical field.

Ioban 2 23x23 Sterile Surgical Drape Non-Fenestrated **Henry Schein Animal Health Catalog #**7774142

Autoclaved surgical tools were used for the procedure. Laparotomy was performed using a disposable scalpel and a monopolar electrocautery device. A ~15 cm skin incision was made using a scalpel. Incision ran along the midline, distal from the navel. In males, the incision line around the navel and distal to it was ~2cm lateral from midline to avoid damage to the urethra. This was followed with dissection of, from superficial to deeper: subcutaneous fat and linea

alba using cautery. The peritoneum was then elevated with forceps and incised sharply to access the peritoneal cavity. The peritoneal incision was widened with cautery, with care taken to avoid injury to underlying viscera.

The cecum was located and a 5-cm purse-string suture (3-0 polydioxanone suture on SH needle, J316H, Ethicon) was placed approximately 5 cm proximal from the ileocecal junction. A longitudinal colotomy was created with cautery through the taenia coli within the purse-string suture. The base of the 3D-printed biocompatible T cannula was inserted into the cecum through this opening. The cannula were made of ABS-M30i material, biocompatible (ISO 10993 USP Class VI), measured 4.5 cm in width (base of the cannula inserted in the cecum), 4.8-5.2 cm in length (exteriorized length 1.8-2.1 cm), and had an internal diameter of 2 cm. All together, with the plug, each cannula weighed between 24.3-25 g. The cannula was then secured (the base/flange in the cecum) by tightening the purse-string suture, around the body/barrel of the cannula.

3-0 polydioxanone suture on SH needle **Ethicon Catalog #**J316H

Cecal cannula ABS-M30i material, biocompatible **Stratasys Direct Manufacturing**

A cylinder of tissue at the marked cannula exit site on the flank (approximately 2 cm in diameter) was excised with cautery. The underlying tissues was gently split by blunt dissection. The fascia was divided with cautery and the flank muscle was dissected bluntly in a muscle-sparing fashion. The cecum was affixed to the abdominal wall in Stamm fashion with 4 circumferential sutures about the cannula site. The cannula lumen was occluded with gauze to minimize contamination of the sterile surgical field and the body of the cannula was delivered through the abdominal wall. The cecum was confirmed to be free of kinks or twists. The sutures were then tied to complete the Stamm approximation. The gauze pack was removed and cannula was capped with a 3D printed cannula cap.

The abdominal cavity was flushed with copious amount of warm saline and suction was used to removed excess fluid. The midline incision was closed in layers. The linea alba, was reapproximated with 0-looped PDS on CTX needle (PDP990G, Ethicon) and the skin was closed in a subcuticular fashion with absorbable suture 2-0 PDS on SH needle (7317H, Ethicon).

0-looped PDS on CTX needle **Ethicon Catalog #**PDP990G

2-0 PDS on SH needle **Ethicon Catalog #**7317H

The sutures skin incision was then covered with skin glue (Vetbond Tissue Adhesive, 3M) and bandaged using Ioban and gauzes. Modified bandaging techniques were used for male pigs to avoid skin irritation due to urine.

Vetbond Tissue Adhesive **3M corporation Catalog #**1469SB

Ioban 2 23x23 Sterile Surgical Drape Non-Fenestrated **Henry Schein Animal Health Catalog #**7774142

Animals were continuously monitored post-surgery until fully awake and ambulatory. Postoperative pain medication was administered in addition to water and food without any postoperative fast.

2.2 Post-operative treatment

Post operative care was provided for 14 days as follows:
Hydromorphone 0.05 - 0.1 mg/kg IV every 4h - first overnight, then Buprenorphine 0.02 mg/kg IV/IM every 12 hours for the initial 48-72 hours post op or Ethiqa SR 0.2 mg/kg SC 4h after the last administration of hydromorphone.
Meloxicam 0.3mg/kg SID (PO/or IM) x 7 days
Excede 5mg/kg IM once 5 days after first injection
Cerenia 2mg/kg SID PO x 1 day
Omeprazole 20mg/day PO x 5 days
Famotidine 1 mg/kg SID x 3 days
Gabapentin 5mg/kg BID PO; give 1 dose day of surgery, and continue x 10 days BID *(12hrs apart)*
Fecal output monitored daily
Cecal cannula assessed and cleaned daily
Surgical incisions assessed daily

- Hydromorphone Hydrochloride Injection, USP **Teva Pharmaceuticals USA, Inc Catalog #**NDC 0703-0113-03
- Ethiqa XR (1.3 mg/ml) **Fidelis Pharmaceuticals Catalog #**NDC 86084-100-30
- Meloxidyl **Zoetis Catalog #**NDC 11695-6966-2
- Excede **Zoetis**
- Cerenia **Zoetis**
- Prilosec OTC (omeprazole 20 mg) **Procter and Gamble Catalog #**NDC 37000-455-02
- Famotidine USP 20 mg **BluePoint Laboratories Catalog #**NDC 68001-397-00
- Gabapentin (oral solution, 250 mg/5 ml) **Amneal Pharmaceuticals LLC Catalog #**NDC 65162-698-90

The low residue diet regimen was continued throughout the post-surgical recovery period (up to 10 days) to insure proper healing of the cecal cannula.

Cecal cannula care

3 The skin surrounding the exteriorization site was cleansed with a dilute Chlorhexidine solution (10% in saline) rinse twice daily as leakage of digesta around the site of exteriorization was occurring occasionally.

Chlorhexidine 2% solution **VetOne Catalog #**11-09007

The cannula sites and surrounding skin were then dried and coated generously with zinc oxide ointment (Desitin, 40% zinc oxide) once or twice daily throughout the study to protect skin from irritation caused by any leaked digesta.

Desitin **Johnson & Johnson Pediatrics**