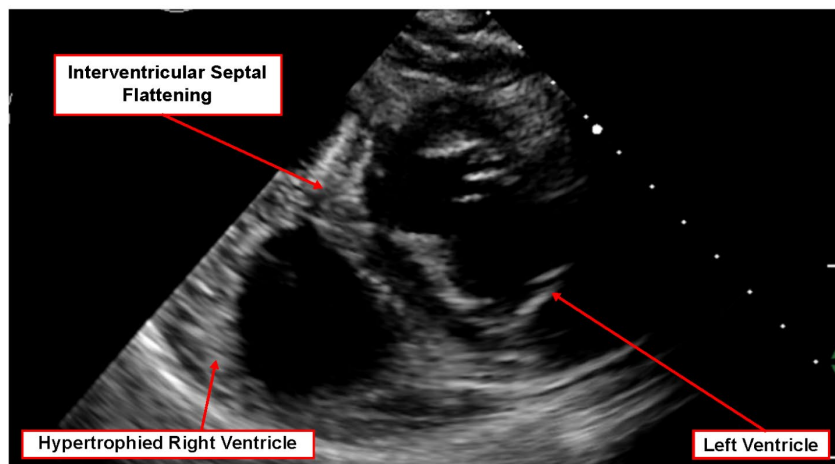
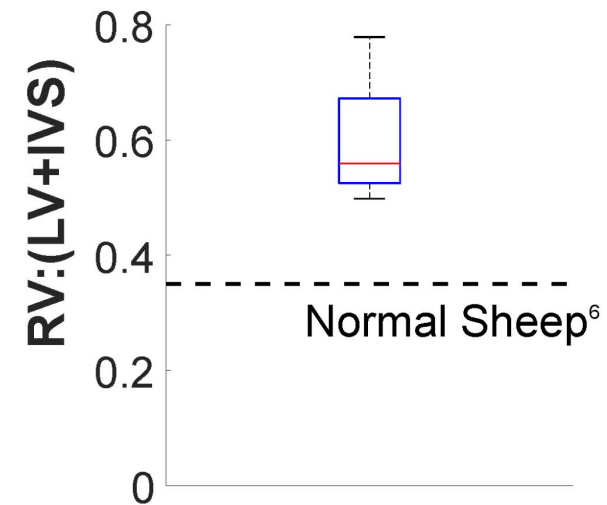
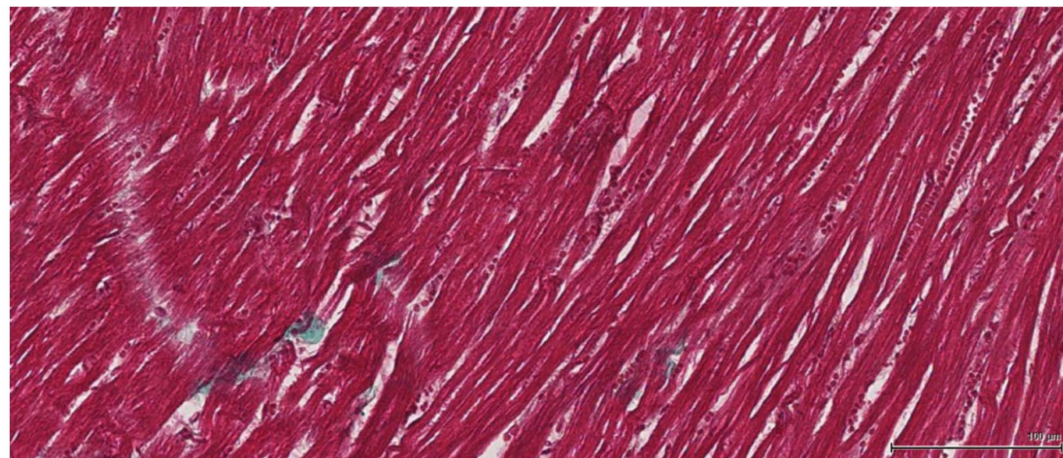


Supplementary Figure 1: Changes in main pulmonary artery cuff pressure over the two-month period of sheep PH-RVH model

**a****b****c****d****Healthy****PH-RVH**

**Supplementary Figure 2:** The evaluation of PH-RVH model: a) the transthoracic echocardiograph of the heart, demonstrating RV hypertrophy and interventricular septal flattening; b) the necropsy of the heart-lung *en bloc* with the pulmonary artery cuff (white arrow); c) the weight ratio between right ventricular free wall and left ventricle + interventricular septum (RV:(LV+IVS)). The data show minimum, maximum, interquartile range, and median of the ratio for 4 animals, and the dotted line represents an average RV:(LV+IVS) value in normal sheep from previous study.<sup>6</sup> d) Gömöri trichrome stain comparing RV tissue between healthy sheep (left) and PH-RVH sheep from the presented model (right).

**Supplementary Method 1:** Step-by-step guide of MPA occluder and RVOT pressure implantations, LPA ligation, and progressive MPA banding

*Preparation of the animal:*

1. Withhold food for 24 hours prior to the procedure to decompress its rumen.
2. Apply a 50 µg/hr fentanyl patch to a shaved area on sheep's dorsum 12 hours before the procedure. Clean the area with chlorohexidine to remove lanolin oil residues prior to patch application. Cover and protect the patch.
3. Administer tiletamine/zolazepam (2.2-5 mg/kg), and apply a facemask with 1-3% isoflurane mixed with 80-100% oxygen for induction.
4. Place sheep supine on the table and restrain its legs.
5. Intubate with 10-mm endotracheal tube and start mechanical ventilation under volume-control mode (10 mL/kg, 15 breaths minute<sup>-1</sup>).
6. Once sedated, insert a 20-gauge angiocatheter in the auricular artery, and obtain baseline arterial blood samples and pressure measurements.
7. Shave the sheep's neck and chest down to upper abdomen.
8. Place 3/8"-1/2" ID orogastric tube for rumen decompression. The orogastric tube will remain in the rumen throughout the entire procedure.
9. Perform fluoroscopy to confirm the heart position and identify the optimal location for the mini-thoracotomy, typically the 4<sup>th</sup> intercostal space.
10. Prep neck and chest with chlorohexidine and betadine solutions and drape the surgical field in sterile fashion.
11. Using ultrasound guidance and Seldinger technique, insert a 7-French triple-lumen central venous catheter (Teleflex; Reading, PA) into the right internal jugular vein for intravenous access and pressure monitoring. Administer 20 mg/kg of cefazolin intravenously. Repeat antibiotic dosing every 4 hours during the procedure.

12. Administer a 10cc/kg bolus of isotonic crystalloid solution to optimize preload before PA ligation. Begin a maintenance intravenous fluid rate of 15cc/kg/hr.

#### Operative procedure

1. Perform a rib-sparing mini-thoracotomy (length<8 cm) at the left fourth intercostal space to obtain mediastinal exposure (**Figure 1a**). A mini-thoracotomy is chosen to expedite post-operative animal recovery.
2. Incise the pericardium anterior to the phrenic nerve and create a pericardial well with 2-0 silk sutures to provide exposure to the main PA and RV.
3. Dissect around the main PA and isolate it with an umbilical tape (**Figure 1b**).
4. Complete intra-pericardial dissection of LPA and encircle it with an umbilical tape.
5. Place a heavy-duty silicone vascular occluder (16–20mm lumen diameter, Docxs Biomedical Products and Accessories, Ukiah, CA) around the main distal PA (**Figure 1c**). Occluder size can be adjusted based on PA diameter.
6. Encircle the proximal main PA with a ¼” Penrose drain to facilitate dissection and maintain spacing for the occluder and subsequent re-operative surgery. Trim the Penrose drain to fit loosely around the PA and secure with an end-to-end 4-0 Prolene suture
7. Prepare RV pressure line: cut off the male end of a 36” pressure tubing at 30° angle to facilitate insertion through the myocardium.
8. Place and secure the cut end of the pressure tubing into the RV outflow tract (RVOT) with a pledgeted 5-0 Prolene suture and silk tie (**Figure 1d**). Measure depth of tubing within the RVOT to ensure tubing position and patency.
9. Connect the opposite end of the tubing to a pressure transducer and monitor to measure baseline RV pressure.
10. Ligate the left PA with umbilical tape (**Figure 1e**). Note the animal’s hemodynamic response to ligation.

11. Inject up to 3 mL of saline into the occluder for testing, while monitoring hemodynamic stability. Once the hemodynamic response is noted, draw back saline and secure the occluder onto the vessel wall with a 5-0 Prolene suture to prevent migration.
12. Tunnel RVOT and occluder tubing out the left dorsum through small incisions and attach them to separate subcutaneous access ports (Access Technologies, Skokie, IL). Anchor the ports subcutaneously with 3-0 Prolene sutures (**Figure 1f**) and close the incisions. Pressure readings are re-confirmed through percutaneous access of the ports.
13. Place a 16-French chest tube in the pleural cavity, secure to the skin, and connect to a closed chest tube drainage unit at a pressure of -20 mmHg.
14. Administer intercostal nerve block (0.25% bupivacaine) with a spinal needle for post-operative analgesia.
15. Close the thoracotomy with interrupted 0 Vicryl sutures. Close soft tissue in layers of running 2-0 Vicryl sutures, and staple the skin.
16. Return animal to dorsal recumbency, remove the orogastric tube, and discontinue isoflurane.
17. Continue mechanical ventilation and supportive care until arterial blood pH>7.35 and pCO<sub>2</sub> < 55 mmHg.
18. Extubate once the animal is breathing spontaneously, lifting its head, and chewing on the endotracheal tube. Remove the chest tube prior to full anesthetic recovery.
19. Transfer the animal to its cage while monitoring its anesthesia recovery. Supplemental oxygen (3-5 L/min by facemask) should be available at all times while the sheep remains immobile. Monitor vital signs every hour for the first 4 hours, every 8 hours for the next 24 hours, and once daily thereafter.

#### Post-operative recovery (3 days)

1. Monitor thoracotomy and port implantation sites daily for signs of infection. Administer long-acting antibiotic (ceftiofur, 5 mg/kg intramuscularly) within 24 hours after the procedure.

2. The fentanyl patch is continued post-operatively for a total of 72 hours. Thereafter, provide additional analgesia (e.g. buprenorphine, 1.5-10 µg/kg every 4-6 hours intramuscularly, or meloxicam, 1 mg/kg once daily intramuscularly) if the animal continues to show signs of pain (i.e. teeth grinding).

Development of PH-RVH (3 days post-operative)

Pressures are recorded using transducers from Edwards Life Science (Irvine, CA) and PowerLab and OCTAL Bio-Amp from ADInstruments (Dunedin, New Zealand).

1. Transfer sheep to a small enclosure. (**Figure 2a**)
2. Shear off excess wool around both subcutaneous ports.
3. Clean shaved areas with 70% alcohol.
4. Apply topical lidocaine spray for local anesthetic.
5. Prepare two transducers for monitoring RV and occluder cuff pressures. Attach one end of the 36-inch pressure tubing to each transducer, and other end to a three-way stopcock. Attach a 22-gauge Huber needle to the stopcock for port access. (**Figure 2b**)
6. Insert needle into the RV port to measure RV pressure. Likewise, insert Huber needle into the occluder port. (**Figure 2c**)
7. Slowly inject up to 1 mL of 3% hypertonic saline into the occluder port in 0.2 mL increments while paying attention to sheep's physiologic response and pressures.
8. Record pressures for several minutes as the sheep relaxes (**Figure 2d**), while noting any changes in its positioning. Any drastic change in cuff pressure over time can indicate cuff leakage (**Supplemental Figure 1**).
9. Remove the needle from the occluder port.
10. Flush the RV port with 10 mL of saline, and lock with 5000 units of heparin.

Repeat steps 1 – 10 every 2 – 4 days while monitoring for changes in heart rate, respiratory rate, and RV and cuff pressures.

## **Supplementary Method 2: The evaluation of PH-RVH**

### **Transthoracic Echocardiography**

Transthoracic echocardiography was performed using Philips Epiq7 ultrasound machine with an X5-1 cardiac probe.

### **Necropsy and Histology**

After euthanasia, heart and lung were excised *en bloc*. From the heart, both right and left atria were separated from the ventricles by cutting along the atrioventricular ring. Right ventricular (RV) free wall was separated from the left ventricle and the interventricular septum (IVS+LV). Each was weighed separately to determine RV:(LV+IVS) ratio. RV free wall tissue specimen was excised transmurally and then fixed in 10% normal-buffered formalin for 24-36 hours. Thereafter, the fixed RV tissue was dehydrated in ethanol, and then processed in xylene. The processed RV tissue was paraffin-embedded and then cut into 5 µm sections. The RV tissue was then stained with Gömöri trichrome.

Slide images were captured using a high throughput Leica SCN400 Slide Scanner automated digital image system from Leica Microsystems. Whole slides were imaged at 20X magnification to a resolution of 0.5 µm/pixel



## Supplementary Tables

**Table S1:** List of surgical instruments

<b>Surgical Instruments</b>	<b>Quantity</b>	<b>SKU # (Mueller)</b>
No. 3 knife handle	1	SU1403-001
No. 7 knife handle	1	SU1407
Adson forceps	2	NL1400
Allis tissue forceps	3	CH1560
DeBakey Aorta clamp	1	CH7247
Aortic clamp, straight (bainbridge forceps)	1	SU6001
U.S.A retractor	2	SU3660
Ferris smith tissue forceps	1	SU2510
Castroviejo needle holder	1	CH8589
Chest tube passer	1	CH04189
Cooley tangential occlusion clamp	1	CH6572
Debakey tissue forceps, 12"	4	CH5906
Debakey vascular tissue forceps, 9"	4	CH5904
Debakey vascular tissue forceps 7 3/4"	4	CH5902
DeBakey multi-purpose clamp	1	CH7276
Finochietto rib spreaders, medium	1	CH1215-1
Finochietto rib spreaders, large	1	CH1220-1
Foerster sponge forceps, curved	2	GL660
Gerald-DeBakey forceps	1	CH04242
Glassman Allis	3	SU6152
Harken clamp	1	CH6462
Mosquito hemostats	4	88-0301
Kelly hemostats	4	88-0314
Flexsteel ribbon retractor, 2" x 13"	1	SU3346
Flexsteel ribbon retractor, 1" x 13"	1	SU3340
Mayo dissecting scissors, curved	1	SU1826
Mayo dissecting scissors, straight	1	SU1821
Sponge bowl	2	GE-75
Vital metzenbaum dissecting scissors, 14"	1	CH2009
Vital metzenbaum dissecting scissors, 9"	1	CH2006
Super cut metzenbaum dissecting scissors	1	CH2032-S
Super cut nelson-metzenbaum dissecting scissors	1	CH2025-S
Halsted mosquito forceps	4	SU2702

Vital crile-wood needle holder, 10-3/8"	1	CH2427
Vital mayo-hegar needle holder, 7-1/4"	1	CH2417
Berry sternal needle holder	1	CH2540
Kantrowitz thoracic clamp, 9-1/2"	1	CH1722
Mixter thoracic forceps, 9"	1	CH1730-003
Schmidt tonsil artery forceps	1	M01700
Lorna non-perforating towel forceps	1	SU2937
Backhaus towel forceps	6	SU2900
Tuffier rib retractor	1	CD1101
Vanderbilt deep vessel forceps	1	CH1687
Vital ryder needle holder, 9"	1	CH2510

**Table S2:** List of surgical disposables

<b>Disposables</b>	<b>Quantity</b>	<b>Supplier, Catalog #</b>
JorVet Sheridan Endotracheal Tube, 10mm	1	Patterson Veterinary, 07-882-9008
Fisherbrand™ Tygon S3™ E-3603 Flexible Tubings *drill multiple holes into the lateral wall to facilitate rumen drainage	1	Fisher Scientific, 14-171-227
Sterile Disposable Deluxe OR Towel, Blue, 17" x 27", 2/Pack	2	Medline, MDT2168202
Three-quarter surgical drape	1	Medline, DYNJP2414H
Skin stapler 35 wide, with counter	2	Medline, STAPLER35W
Sponge, Lap: X-Ray Detectable Sterile Lap Sponge, 18" x 18", 5/Pack	2 packs	Medline, MDS241518HH
Blades: Stainless-Steel Sterile Surgical Blade, Size #11	1	Medline, B-D371211
Blades: Stainless-Steel Sterile Surgical Blade, Size #15	1	Medline, B-D371215
Blades: Stainless-Steel Sterile Surgical Blade, Size #10	1	Medline, B-D371210
Electrodes: Valleylab REM Polyhesive Cordless Patient Return Electrodes, Adult	1	Medline, SWDE7509
Valleylab Button Switch Pencil	1	Medline, VALE2516H
Blades, Electrode: Electrode Blade, 6.5", with 0.24 cm Shaft	1	Medline, VALE15516

Tubing, Suction: Sterile Universal Suction Tubing with Straight Ribbed Connectors, 1/4" x 12'	1	Medline, OR612
Yankauer, Bulb Tip: Sterile Rigid Yankauer with Bulb Tip, No Vent	1	Medline, DYND50130
Gauze Sponges: Sterile X-ray Compatible Gauze Sponges, 16-Ply, 4" x 4"	1	Medline, PRM21430LFH
0.9% Sodium Chloride Irrigation Pour Bottle by Baxter Healthcare, 1000 mL	1	Medline, BHL2F7124
Perma-Hand Suture, Black Braided, Size 0, 6 x 30"	1	Medline, ETHA306H
Perma-Hand Suture, Black Braided, Size 4-0, 12 x 30"	1	Medline, ETHA303H
PROLENE Monofilament Suture, Blue, Size 4-0, 36", Double Arm, RB-1 Needle	1	Medline, ETHD7143
PROLENE Polypropylene Monofilament Suture, Blue, Double-Armed, RB-1 Needle, Size 5-0, 24"	2	Medline, ETH8555H
Perma-Hand Black Braided Silk: 2-0 SH Taperpoint Needle, Control Release, 30"	1	Medline, ETHD8552
Vicryl: Undyed Coated Vicryl 2 TP-1 Taper 54" Suture	2	Medline, ETHVCP880T
Vicryl: Undyed Coated Vicryl 0 CT-1 36" Suture	2	Medline, ETHVCP946H
Vicryl: Undyed Coated Vicryl 2-0 CT-1 18" Suture	2	Medline, ETHVCP739D
Loop, Vessel, Mini, Red, 2/pk, Sterile	2	Medline, DYNJVL12
Umbilical Tape, Cotton, 3-Strand, 1/8 x 36"	2	Medline, ETHU12TH
TourniKwik Tourniquet Set with Four 7.5" Bronze-Colored Tubes and 1 Snare, 12 French	1	Medline, CVR79013
Syringes: Sterile Luer-Lock Syringe, 10 mL	3	Medline, SYR110010Z
Needles: Hypodermic Needle with Regular Bevel, Sterile, 18 G x 1.5"	1	Medline, B-D305185Z
BD Regional Block Needles, 22-gauge	1	Medline, B-D408348Z
Ligaclip Multiple-Clip Appliers by Ethicon	1	Medline,

		ETHMCS20
20mm Heavy Duty Occluder with actuating tubing	1	Access Technologies, OC-20HD
Argyle Penrose Tubing by Cardinal Health, 6 mm x 46 cm, 11 mm Flat	1	Medline, SWD514604H
Large CP2 Port with 5-French Connector and Blue Boot (Subcutaneous port)	2	Access Technologies, CP2AC-5NC
Argyle Thoracic Catheter, Straight, 28 Fr x 20"	1	Medline, SWD570549H
Pleur-evac A-6000 Series Chest Drain, with Dry Suction, Adult-Pediatric	1	Medline, DEKA6000LFH
Medipore Dress-It Pre-Cut Dressing Covers by 3M	2	Medline, MMM2955Z
Multi-Lumen Central Venous Catheterization Kits by Teleflex	1	Medline, ARW45703XP1AH
Sterile Surgical Equipment Probe Covers	1	Medline, DYNJE5930
Skin Prep Applicator: Chloraprep 3-mL Skin Prep Applicator	2	Medline, MDF260400
Tubing: Pressure Monitoring Tubing with Fixed Male Luer Lock and Female Fitting, Low Pressure, 72" L	2	Medline, DYNJPMTBG72MF
TruWave Disposable Pressure Transducer Kits by Edwards Lifesciences	2	Medline, VSYPX260
Stopcock: 3-Way Stopcock with Handle in OFF Position, Rotating Adaptor Male Collar Fitting, 45 PSI	2	Medline, DYNJSC301
Sponge, Peanut: X-Ray Detectable Sterile Peanut Sponge, Small, 3/8"	1	Medline, MDS72038

**Table S3:** List of surgical monitoring equipment

Monitoring Equipment	Quantity	Manufacturer, Model Name or Number
Data Acquisition Device	1	ADInstruments, PowerLab 16/30

Bridge Amplifier	1	ADInstruments, FE228
Vital Signs Monitor	1	Smiths Medical, SurgiVet® Advisor® 3
Veterinary Anesthesia Ventilator	1	Hallowell EMC, Model 2000
Veterinary Anesthesia Machine	1	Midmark, Matrx VMC
Intravenous Pump	3	Heska, Vet/IV 2.2 Infusion Pump
Electrosurgical Generator	1	Covidien, Force FX-C
Heat Therapy Pump	1	Gaymar/Stryker, TP-400

**Table S4:** List of pre-, intra-, post-operative, and euthanasia medications

Pre-Op		
Medication (Manufacturer, NDC #)	Route	Dose
Fentanyl (Apotex Corp, 60505-7007-2)	Transdermal	50 mcg/hr
Tiletamine + Zolazepam (Zoetis Inc, 54771-9050-1)	Intramuscular	4 mg/kg
Isoflurane (Patterson, 14043-704-06)	Inhaled	1-5%
Ophthalmic Ointment (Akorn Animal Health, 59399-162-35)	Topical	N/A

Intra-Op		
Medication (Manufacturer, NDC #)	Route	Dose
Cefazolin (Apotex Corp, 60505-6142-0)	Intravenous	20 mg/kg
Enrofloxacin (Norbrook Laboratories Limited, 55529-152-05)	Intravenous	5 mg/kg
0.25% Bupivacaine (Hospira Inc, 0409-1160-18)	Intercostal	1 mg/kg

0.9% normal saline, 1000 mL (Baxter Healthcare Corp, 0338-0049-04)	Intravenous	1-10 mL/kg/hr
Phenylephrine (West-Ward, 0641-6142-25)	Intravenous	1-5 µg/kg/min

Post-Op		
Medication (Manufacturer, NDC #)	Route	Dose
Ceftiofur Crystalline Free Acid (Zoetis, 54771-5223-1)	Intramuscular	5 mg/kg
Meloxicam* (Patterson Veterinary, 14043-909-10)	Intramuscular	1 mg/kg
Buprenorphine (Reckitt Benckiser, 12496-0757-1)	Intramuscular	1.5-10 µg/kg

Euthanasia		
Medication (Manufacturer, NDC #)	Route	Dose
Pentobarbital sodium (Patterson Veterinary, 51311-050-01)	Intravenous	>120 mg/kg

**Table S5:** List of disposable items for chronic PA banding procedure

Item	Quantity	Supplier, Catalog #
TruWave Disposable Pressure Transducer Kits by Edwards Lifesciences	2	Medline, VSYX260
Tubing: Pressure Monitoring Tubing with Fixed Male Luer Lock and Female Fitting, Low Pressure, 72" L	2	Medline, DYNJPMTBG72MF
Bags, Infusion: Nonsterile Novaplus Infusion Bag, 500 mL	1	Medline, ETCV4005H
Sterile Luer-Lock Syringe, 5 mL	1	Medline, SYR105010Z
Sterile Luer-Lock Syringe, 3 mL	1	Medline, SYR103010Z

Hypodermic Needle with Bevel and Regular Wall, 20 G x 1"	2	Medline, B-D305175Z
Stopcock: 3-Way Stopcock with Handle in OFF Position, Rotating Adaptor Male Collar Fitting, 45 PSI	2	Medline, DYNJSC301
Port-A-Cath Huber Needle, Straight, 22 G x 1-1/2"	2	Medline, AAKM21200724

**Table S6:** List of medications during chronic PA banding procedure

<b>Medication (Manufacturer, NDC #)</b>	<b>Route</b>	<b>Dose</b>
Hypertonic saline 3% (Baxter Healthcare Corp., 0338-0054-03)	Occluder port	Up to 1 mL
Heparin (Fresenius Kabi, 63323-540-31)	RV port	5000 units
Lidocaine HCl, 2.46% (PRN Pharmacal, 49427-434-04)	Topical	Cover port area
0.9% normal saline, 500 mL (Baxter Healthcare Corp., 0338-0049-03)	RV port	10 mL

**Table S7:** Humane endpoint criteria for the presented PH-RVH model

<ul style="list-style-type: none"> <li>• Body weight loss of greater than 20% compared to baseline, pre-surgical body weight</li> <li>• Mean arterial blood pressure &lt;60mmHg that persists over 48 hours that cannot be corrected within a period of 4 hours</li> <li>• Anemia (Hg &lt; 6 g/dL)</li> <li>• Uncontrolled pain (teeth grinding)</li> <li>• Inability to stand or eat for more than 24 hours despite veterinary intervention</li> <li>• Repeated, long-term ramming of head for &gt;48 hours</li> <li>• Constant vocalization for &gt;48 hours</li> </ul>
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