PROTOCOL FOR QUICK NON-FORENSIC POST-MORTEM EXAMINATION THAT CAN BE CARRIED OUT IN GENERAL PRACTICE

INTRODUCTION:

Establishing the cause of death in a rabbit requires a post-mortem examination. Whilst this can be carried out by an external laboratory, rabbit tissues autolyse very quickly - ideally they need to be collected within 6 hours of death. Even refrigerating a rabbit does not prevent this autolysis.

- RHDV2 kills rabbits extremely quickly with very few clinical signs.
- It is very infectious.
- A PCR test will confirm the presence of the virus but requires fresh liver to give a reliable result.
    - If the PCR is negative then we need more information and a post mortem examination is the only way we can obtain this.
- A post-mortem examination combined with histology on a range of tissues is a reliable method of diagnosing RHDV but will also uncover other problems if they are present.
- A full post-mortem examination is very time-consuming and deconstructive therefore this protocol was devised.

The following protocol has been developed over the years by Nigel and Frances Harcourt-Brown. It allows for a rapid but relatively thorough examination and the collection of a standard set of tissues.

HOW LONG WILL IT TAKE AND WHY DO A POST MORTEM EXAMINATION?

- It should take 20 minutes and some of this time is spent repairing the incision
- Autolysis is kept to a minimum if it is done quickly and in house.
- The clinician in charge can carry out the examination, which is beneficial.
- The results of the gross examination are available immediately.
- The cost of this in-house examination can be kept to a minimum.

- The histology fees are usually similar to the cost of a PCR test but can tell the veterinarian much more. Visit www.nwlabbey.co.uk for more information.
- Also, if the samples are sent to Abbey Veterinary Services, they will allow up to 4 samples per histological exam to be covered in the fee. The histopathologists are willing to look at a range of tissues.
- **Remember:** It is possible to repair the incision and the rabbit can be returned to the owner for burial etc. Many owners will allow a PME if they can have their pet returned but will refuse PME if they cannot have the animal back.

POST-MORTEM EXAMINATION REQUIREMENTS:

- Mayo scissors, scalpel handle and blade, toothed dissecting forceps, needle holders, needle and suture material (monofilament nylon).
- Ideally a large container that will hold 50- 100mls of formol saline (big tablet pot or tupperware box).
- Protective gown, gloves.
- Disinfection protocol for the post-mortem examination area.
- A camera at hand to take any pictures.
    - Why not use your smart phone? A sealed plastic bag can be used to protect it (you may need to cut a section out for the lens to see through).

PROTOCOL

The tissues that need to be collected and submitted for histology are:

- Heart and lungs (whole organs).
- Piece of liver.
- Whole kidney.
- Piece of, or whole, spleen.

- Make a midline skin incision FIG A from throat to pubis and expose the musculature beneath.
- Free some of the skin from the musculature so that it is away from the incision. This prevents hair entering the body cavity and blood contaminating the fur.

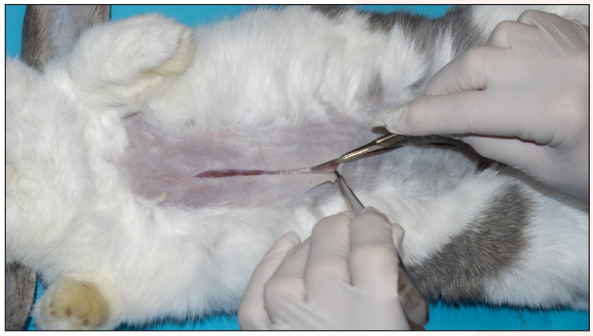

FIG A - SMALL NICK IN ABDOMEN

- To open the abdomen FIG B, tent the skin for the initial small nick so that air can enter and the linea alba can be cut without opening the caecum. Extend the incision from xiphisternum to pubis. It can be helpful to incise the muscles following the line of the ribs and pubic brim FIG C. This allows the muscles to be folded back to expose more of the viscera without letting them displace too much. However, it is more difficult to repair the incision at the end of the procedure.

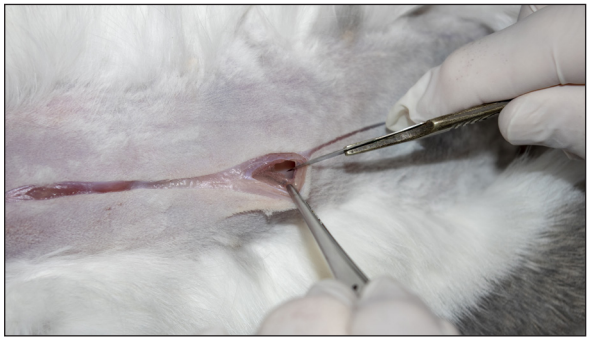

FIG B - OPEN ABDOMINAL CAVITY

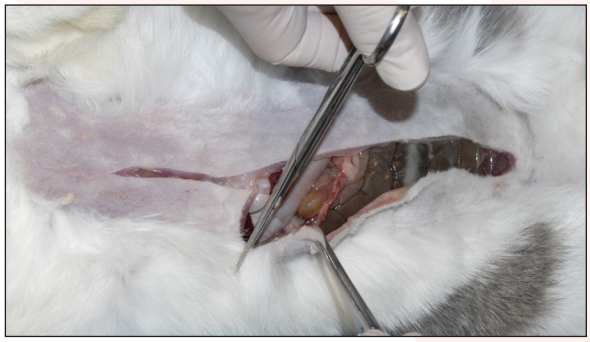

FIG C - OPEN ABDOMEN

- Look at the gross appearance of the viscera FIG D and presence of any fluid, blood or ingesta in the abdominal cavity.
- Is the stomach distended and filled with fluid and gas? If so, look for a small intestinal obstruction especially at the pyloric end of the duodenum. The common causes of obstruction are pellets of impacted fur or neoplasia. Gastric dilation can also be a sign of mucoid enteropathy or paralytic ileus.

FIG D - ABDOMINAL CONTENTS

- Examine the liver FIG E and assess its size and colour. Take representative samples (about 2cm^2 of tissue) and place them in the formol saline for histopathology. Collect two pieces of liver to be stored in the freezer in case PCR testing for RHD is required. Frozen samples do not need fixing. One can be sent to either PALS or BattLab. The other sample can be retained in case the first was lost in transit or you require repeat tests.

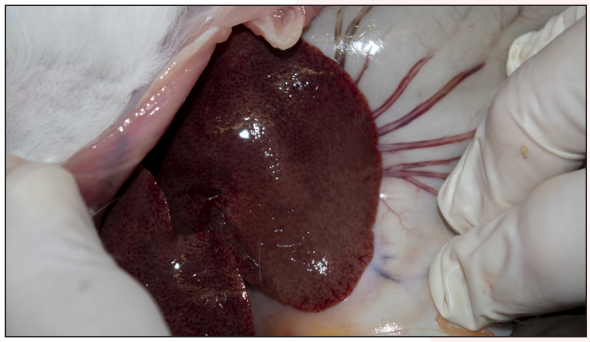

FIG E - LIVER

  - Make sure to check the whole liver. The caudal part of the caudate lobe is the main area that will be affected by a fatal liver tortion (see fig H).

- Lift the stomach and examine the spleen FIG F attached to the stomach by lesser omentum. Remove at least half the spleen and put into the formol saline.

- Move the guts to the right and find the left kidney FIG G, H (and adrenal gland) Note the size and surface of the kidney. Do the same on the other side. Remove at least one kidney, make a sagittal cut through the organ and put into formol saline.

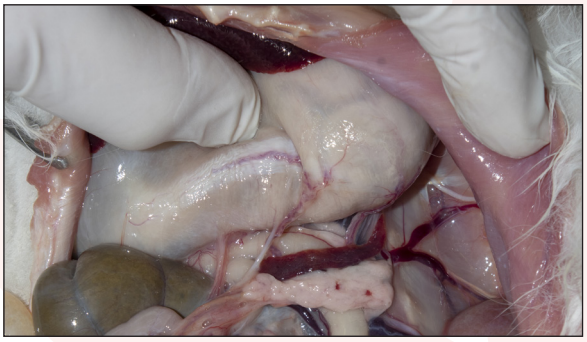

FIG F - REFLECT STOMACH TO REVEAL SPLEEN

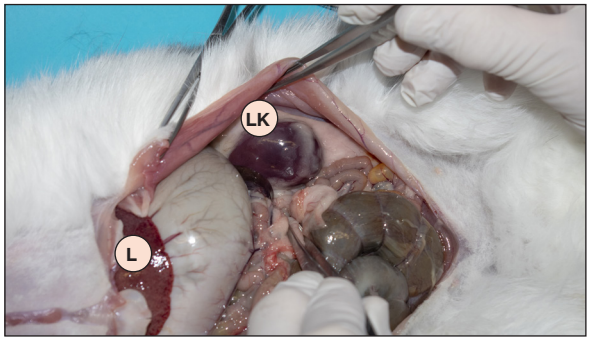

FIG G - LEFT KIDNEY (LK) AND LIVER (L)

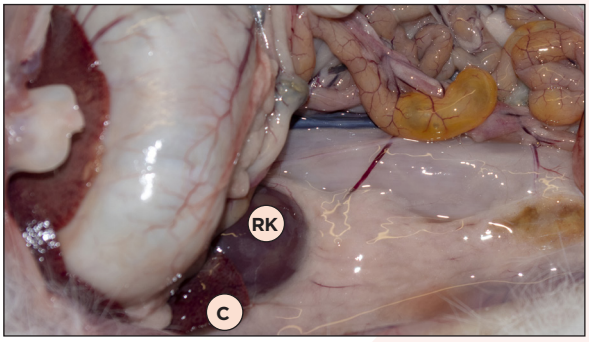

FIG H - RIGHT KIDNEY (RK) OVERLAIN BY THE CAUDATE PART OF THE CAUDAL LOBE OF THE LIVER (C)

- Examine the bladder FIG I. If it is a neutered female, look for adhesions between the bladder and the uterine/vaginal stump. It is an opportunity to see the results of surgery.

- Make a small nick in the diaphragm to allow the lungs to move away from the ventral thoracic wall and then incise along one or both sides of the sternum FIG J.

- Retract the ribs and sternum to open the chest cavity. Note the external surface of heart and pericardium. Look at all the lungs *in situ*.

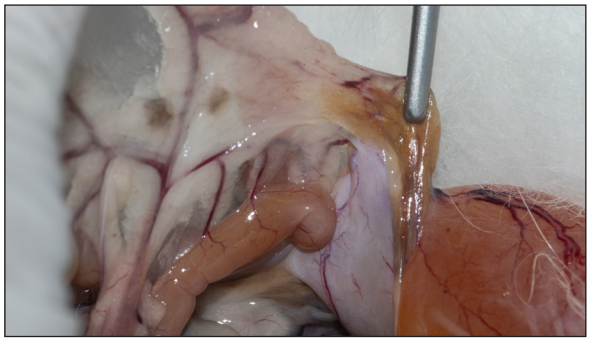

FIG I - ADHESION: VAGINA TO RECTUM AND BLADDER

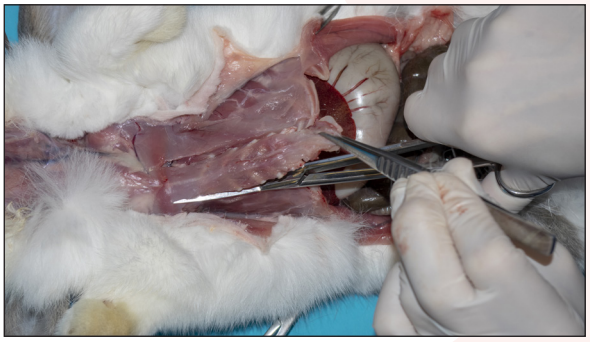

FIG J - OPEN CHEST

- Incise up the neck to expose the trachea FIG K. Open the trachea to the pharynx to check for the presence of a foreign body. Note: The internal surface of the trachea in rabbits is naturally hyperaemic and red in colour.
- Section the trachea and lift/dissect out the pluck FIG L. Examine the heart and lungs grossly and preferably gently palpate the lungs FIG M.

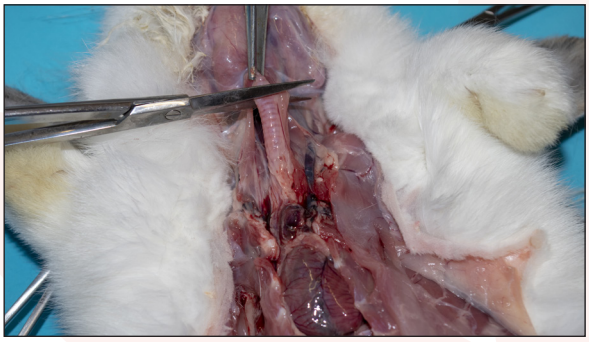

FIG K - SECTION TRACHEA

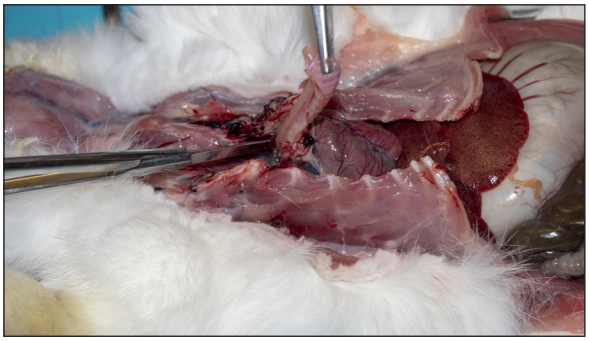

FIG L - REMOVE PLUCK

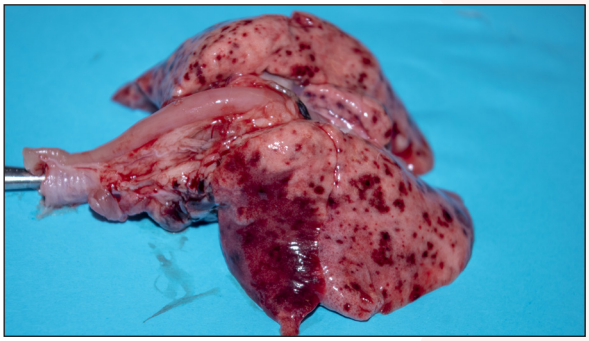

FIG M - PLUCK: ABNORMAL LUNGS

- Put the whole pluck into the formol saline but keep the cut edge of the trachea held in forceps and trickle formol saline into the trachea FIG N using a pipette. Three to 5 mls is all that is needed to fix the lungs internally. Tie a ligature around the trachea.
- Preferably, inject a couple of mls of formol saline into the chambers of the heart, especially if the heart is large and the pericardial sac if it is not opened. Then immerse the whole pluck in formol saline.
- Repair the incision. Paper towelling can be used to pack the chest cavity and absorb any blood FIG O.

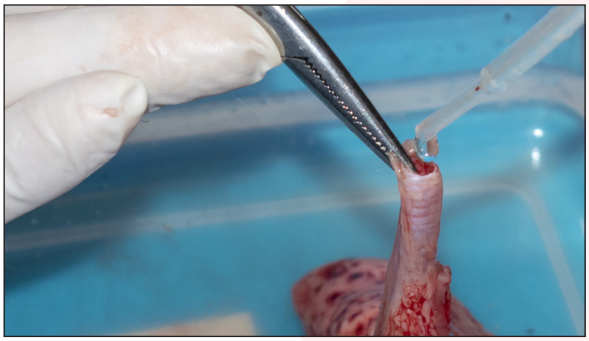

FIG N - FILL LUNG WITH F-SALINE

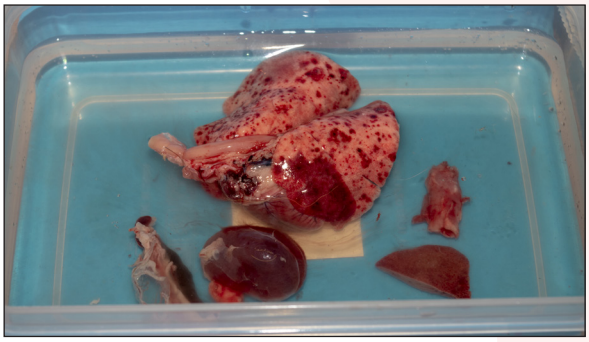

FIG O - FINISHED

Once the tissues for histopathology have fixed (more than 48 hours), the formol saline can be drained off and the fixed tissues placed in a sealable plastic bag. This bag is then surrounded by paper towels, placed into another sealable bag and finally a padded envelope. This seems to be sufficient protection for the tissues. Post fixed samples to the lab of your choice ensuring you have checked their pricing structure. A lab will either have a set PME histology fee or a fee per tissue sample submitted.

ADDITIONAL TISSUES

The histopathologist is willing to look at any additional tissues that are submitted. If any abnormal tissue is found during post-mortem examination, it can be included and sent with the rest of the samples.

Intestinal pathology

- Due to autolysis, meaningful results can only be gained from intestinal tissue if it is really fresh (i.e within 1 hour of death).
- It is often helpful to submit samples from different areas of the gastrointestinal tract. They need to be labelled so the histopathologist knows which parts the samples came from.
- To take a sample of intestine – isolate the length of interest and tie off each end with sutures. Inject sufficient formol saline to distend the gut and place into the pot with the other samples. It is sensible to tie off the ends of the gut that remain within the carcase.
- Remove and place in formol saline.
- Alternatively, open a length of gut and place it, peritoneal side down, on a section of a wooden tongue depressor. If need be, write in pencil on the wood prior to fixing the tissue onto it.

CNS tissue

Collection of CNS tissue is time-consuming and unless there are any obvious gross lesions, the results can be disappointing.